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DOCKET NO. : TJU-2444

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of: Scott A. Waldman

Serial No.:

Group Art Unit:

Filing Date: Herewith

Examiner:

For: ST RECEPTOR BINDING COMPOUNDS AND METHODS OF USING THE SAME

EXPRESS MAIL LABEL NO: EL531172596US

DATE OF DEPOSIT: November 28, 2000

Box ☒ Patent Application  
☐ Provisional ☐ Design

Assistant Commissioner for Patents  
Washington DC 20231

Sir:

PATENT APPLICATION TRANSMITTAL LETTER

Transmitted herewith for filing, please find

☒ A Utility Patent Application under 37 C.F.R. 1.53(b).

It is a continuing application, as follows:

☐ continuation ☒ divisional ☐ continuation-in-part of prior application number  
08/468,449, which is a divisional of application number 08/141,892.

☐ A Provisional Patent Application under 37 C.F.R. 1.53(c).

☐ A Design Patent Application (submitted in duplicate).

Including the following:

☐ Provisional Application Cover Sheet.



09724983-112800

- ☐ New or Revised Specification, including pages \_ to \_ containing:
- ☐ Specification
  - ☐ Claims
  - ☐ Abstract
  - ☐ Substitute Specification, including Claims and Abstract.
- ☐ The present application is a continuation application of Application No. \_\_\_\_\_ filed \_\_\_\_\_. The present application includes the Specification of the parent application which has been revised in accordance with the amendments filed in the parent application. Since none of those amendments incorporate new matter into the parent application, the present revised Specification also does not include new matter.
- ☐ The present application is a continuation application of Application No. \_\_\_\_\_ filed \_\_\_\_\_, which in turn is a continuation-in-part of Application No. \_\_\_\_\_ filed \_\_\_\_\_. The present application includes the Specification of the parent application which has been revised in accordance with the amendments filed in the parent application. Although the amendments in the parent C-I-P application may have incorporated new matter, since those are the only revisions included in the present application, the present application includes no new matter in relation to the parent application.
- ☒ A copy of earlier application Serial No. 08/141,892 Filed October 26, 1993, including Specification, Claims and Abstract (pages 1 - 103), to which no new matter has been added TOGETHER WITH a copy of the executed oath or declaration for such earlier application and all drawings and appendices. Such earlier application is hereby incorporated into the present application by reference.
- ☐ Please enter the following amendment to the Specification under the Cross-Reference to Related Applications section (or create such a section) :
- ☐ Signed Statement attached deleting inventor(s) named in the prior application.
- ☒ A Preliminary Amendment.
- ☐ \_\_\_\_\_ Sheets of ☐ Formal ☐ Informal Drawings.

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- ☐ Petition to Accept Photographic Drawings.
- ☐ Petition Fee
- ☐ An ☐ Executed ☐ Unexecuted Declaration or Oath and Power of Attorney.
- ☐ An Associate Power of Attorney.
- ☐ An ☐ Executed ☐ Copy of Executed Assignment of the Invention to \_\_\_\_\_
- ☐ A Recordation Form Cover Sheet.
- ☐ Recordation Fee - \$40.00.
- ☒ The prior application is assigned of record to Thomas Jefferson University.
- ☐ Priority is claimed under 35 U.S.C. § 119 of Patent Application No. \_\_\_\_\_ filed \_\_\_\_\_ in \_\_\_\_\_ (country).
- ☐ A Certified Copy of each of the above applications for which priority is claimed:
- ☐ is enclosed.
- ☐ has been filed in prior application Serial No. \_\_\_\_\_ filed \_\_\_\_\_.
- ☒ An ☒ Executed or ☐ Copy of Executed Earlier Statement Claiming Small Entity Status under 37 C.F.R. 1.9 and 1.27
- ☐ is enclosed.
- ☒ has been filed in prior application Serial No. 08/141,892 filed October 26, 1993, said status is still proper and desired in present case.
- ☐ Diskette Containing DNA/Amino Acid Sequence Information.
- ☐ Statement to Support Submission of DNA/Amino Acid Sequence Information.
- ☒ The computer readable form in this application, is identical with that filed in Application Serial Number 08/141,892, filed October 26, 1993. In accordance with 37 CFR 1.821(e), please use the ☐ first-filed, ☐ last-filed or ☒ only computer readable form filed in that application as the computer readable form for the instant application. It is understood that the Patent and Trademark Office will make the

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## FEE CALCULATION:

- ☒ Cancel in this application original claims 2-21 of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

				SMALL ENTITY		NOT SMALL ENTITY	
				RATE	FEE	RATE	FEE
PROVISIONAL APPLICATION				\$75.00	\$	\$150.00	\$
DESIGN APPLICATION				\$160.00	\$	\$320.00	\$
UTILITY APPLICATIONS BASE FEE				\$355.00	\$355.00	\$710.00	\$
UTILITY APPLICATION; ALL CLAIMS CALCULATED AFTER ENTRY OF ALL AMENDMENTS							
	No. Filed	No. Extra					
TOTAL CLAIMS	28- 20 =	8		\$9 each	\$72	\$18 each	\$
INDEP. CLAIMS	3- 3 =	0		\$40 each	\$0	\$80 each	\$
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				\$135	\$0	\$270	\$
ADDITIONAL FILING FEE					\$		\$
TOTAL FILING FEE DUE					\$427.00		\$

- ☒ A Check is enclosed in the amount of \$427.00.
- ☒ The Commissioner is authorized to charge payment of the following fees and to refund any overpayment associated with this communication or during the pendency of this application to deposit account 23-3050. This sheet is provided in duplicate.
- ☐ The foregoing amount due.
- ☒ Any additional filing fees required, including fees for the presentation of extra claims under 37 C.F.R. 1.16.
- ☒ Any additional patent application processing fees under 37 C.F.R. 1.17 or 1.20(d).
- ☐ The issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance.
- ☒ The Commissioner is hereby requested to grant an extension of time for the appropriate length of time, should one be necessary, in connection with this filing or

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Date: Nov 28 2000



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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

**Scott A. Waldman**

Serial No.:

Group Art Unit:

Filed: **Herewith**

Examiner:

For: **ST RECEPTOR BINDING COMPOUNDS AND METHODS  
OF USING THE SAME**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

**PRELIMINARY AMENDMENT**

This preliminary amendment is being filed with a divisional application filed herewith pursuant to 37 CFR 1.53(b) of pending application Serial Number 08/468,449. Please amend the application as follows.

**IN THE SPECIFICATION:**

On page 1, between the "Title" and the section entitled "**FIELD OF THE INVENTION**" please insert:

**--CROSS REFERENCE TO RELATED APPLICATIONS**

This application is a Divisional application of Serial Number 08,468,449, filed June 6, 1995, pending, which is a Divisional application of Serial Number 08/141,892 filed October 26, 1993, which is U.S. Patent No. 5,518,888 issued May 21, 1996.

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**ACKNOWLEDGMENT OF GOVERNMENT RIGHTS**

This invention was made with Government support under grant number DK43805-01A2 awarded by the National Institutes of Health. The Government has certain rights in this invention.--

**IN THE CLAIMS:**

Please cancel claim 1 and add new claims 22-49 as follows.

**23.** A method of treating an individual suspected of suffering from colorectal cancer comprising the step of administering to said individual a therapeutically effective amount of a pharmaceutical composition that comprises:

- a) an ST receptor ligand;
- b) an active agent, wherein the active agent causes cell death; and
- c) a pharmaceutically acceptable carrier or diluent.

**24.** The method of claim 23 wherein said ST receptor ligand is a peptide.

**25.** The method of claim 24 wherein said ST receptor ligand is a peptide selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NOS:5-54 and fragments and derivatives thereof.

26. The method of claim 25 wherein said ST receptor ligand is a peptide selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NOS:5-54.
27. The method of claim 23 wherein said ST receptor ligand is an antibody, FAb or F(Ab)<sub>2</sub>.
28. The method of claim 27 wherein said ST receptor ligand is an antibody.
29. The method of claim 23 wherein said active agent is a cytotoxic agent.
30. The method of claim 29 wherein said active agent is selected from the group consisting of methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, *cis*-platinum, vindesine, mitomycin, bleomycin, purothionin, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, *Pseudomonas* exotoxin, diphtheria toxin, *Clostridium perfringens* phospholipase C, bovine pancreatic ribonuclease, pokeweed antiviral protein, abrin, abrin A chain, cobra venom factor, gelonin, saporin, modeccin, viscumin, volkensin, nitroimidazole, metronidazole and misonidazole.

31. The method of claim 30 wherein said ST receptor ligand is an antibody, FAb or F(Ab)<sub>2</sub>.

32. The method of claim 29 wherein said ST receptor ligand is an antibody, FAb or F(Ab)<sub>2</sub>.

33. The method of claim 23 wherein said ST receptor ligand is a peptide and said active agent is a cytotoxic agent.

34. The method of claim 33 wherein said ST receptor ligand is a peptide selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NOS:5-54 and fragments and derivatives thereof and said active agent is a cytotoxic agent selected from the group consisting of methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, *cis*-platinum, vindesine, mitomycin, bleomycin, purothionin, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, *Pseudomonas* exotoxin, diphtheria toxin, *Clostridium perfringens* phospholipase C, bovine pancreatic ribonuclease, pokeweed antiviral protein, abrin, abrin A chain, cobra venom factor, gelonin, saporin, modeccin, viscumin, volkensin, nitroimidazole, metronidazole and misonidazole.

35. The method of claim 34 wherein said ST receptor ligand is a peptide selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NOS:5-54.
36. The method of claim 23 wherein said pharmaceutical composition is administered intravenously.
37. A pharmaceutical composition that comprises:
- a) an ST receptor ligand;
  - b) an active agent, wherein the active agent causes cell death; and
  - c) a pharmaceutically acceptable carrier or diluent.
38. The pharmaceutical composition of claim 37 wherein said ST receptor ligand is a peptide.
39. The pharmaceutical composition of claim 38 wherein said ST receptor ligand is a peptide selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NOS:5-54 and fragments and derivatives thereof.
40. The pharmaceutical composition of claim 39 wherein said ST receptor ligand is a peptide selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NOS:5-54.

41. The pharmaceutical composition of claim 37 wherein said ST receptor ligand is an antibody, FAb or F(Ab)<sub>2</sub>.

42. The pharmaceutical composition of claim 41 wherein said ST receptor ligand is an antibody.

43. The pharmaceutical composition of claim 37 wherein said active agent is a cytotoxic agent.

44. The pharmaceutical composition of claim 43 wherein said active agent is selected from the group consisting of methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, *cis*-platinum, vindesine, mitomycin, bleomycin, purothionin, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, *Pseudomonas* exotoxin, diphtheria toxin, *Clostridium perfringens* phospholipase C, bovine pancreatic ribonuclease, pokeweed antiviral protein, abrin, abrin A chain, cobra venom factor, gelonin, saporin, modeccin, viscumin, volkensin, nitroimidazole, metronidazole and misonidazole.

45. The pharmaceutical composition of claim 44 wherein said ST receptor ligand is an antibody, FAb or F(Ab)<sub>2</sub>.



46. The pharmaceutical composition of claim 43 wherein said ST receptor ligand is an antibody, FAb or F(Ab)<sub>2</sub>.

47. The pharmaceutical composition of claim 37 wherein said ST receptor ligand is a peptide and said active agent is a cytotoxic agent.

48. The pharmaceutical composition of claim 47 wherein said ST receptor ligand is a peptide selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NOS:5-54 and fragments and derivatives thereof and said active agent is a cytotoxic agent selected from the group consisting of methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, *cis*-platinum, vindesine, mitomycin, bleomycin, purothionin, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, *Pseudomonas* exotoxin, diphtheria toxin, *Clostridium perfringens* phospholipase C, bovine pancreatic ribonuclease, pokeweed antiviral protein, abrin, abrin A chain, cobra venom factor, gelonin, saporin, modeccin, viscumin, volkensin, nitroimidazole, metronidazole and misonidazole.

49. The pharmaceutical composition of claim 48 wherein said ST receptor ligand is a peptide selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NOS:5-54.

**REMARKS**

This application is a divisional application of pending application Serial Number 08/468,449, filed June 6, 1995.

Upon filing this divisional application, claims 2-22 were canceled prior to calculation of the filing fee.

Accordingly, claim 1 is pending in the application.

By way of this preliminary amendment, claim 1 has been canceled and new claims 23-49 have been added. The specification has been amended to identify and reflect the status of the parent applications.

Upon entry of this amendment, claims 23-49 will be pending.

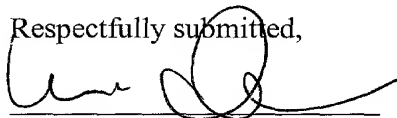
Claims 23-49 have been added to define specific aspects and embodiments of the present invention. Support for new claims 23-49 is found throughout the specification, particularly, pages 42-43. No new matter is added.

Claims 23-49 are in condition for allowance. A Notice of Allowance is earnestly solicited.

Date: Nov 28, 2000

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Respectfully submitted,



**Mark DeLuca**

Registration No. 33,229

**ST RECEPTOR BINDING COMPOUNDS AND METHODS OF USING THE SAME****FIELD OF THE INVENTION**

The present invention relates to compounds which comprise a receptor ligand moiety conjugated to an active agent. More particularly, the present invention relates to compounds which comprise a moiety that binds to the ST receptor conjugated to a therapeutic or imaging moiety.

**BACKGROUND OF THE INVENTION**

Colorectal cancer is the third most common neoplasm worldwide and the second most common in the United States, representing about 15% of the newly diagnosed cases of cancer in the United States. The large intestine or large bowel is the third leading site for the development of new cancer and is diagnosed in about 150,000 patients each year. Colorectal cancer is the second leading cause of cancer-related deaths and is responsible for about 12% of cancer deaths in the United States. The mortality rate of newly diagnosed large bowel cancer approaches 50% and there has been little improvement over the past 40 years. Most of this mortality reflects local, regional and distant metastases. About thirty percent of patients with colorectal cancer have unresectable disease at presentation and about 40% develop metastases during the course of their disease. Distant metastatic disease is seen in liver (about 12%), lung (about 3%), bone (about 0.9%), brain (about 0.7%), nodes (about 4%), and peritoneum (about 2%) at the time of initial diagnosis. In 1987, the large bowel cancers found

regionally or at distant sites at the time of diagnosis were about 26% and about 18%, respectively.

Surgery is the mainstay of treatment for colorectal cancer but recurrence is frequent. Colorectal cancer has proven resistant to chemotherapy, although limited success has been achieved using a combination of 5-fluorouracil and levamisole. Surgery has had the largest impact on survival and, in some patients with limited disease, achieves a cure. However, surgery removes bulk tumor, leaving behind microscopic residual disease which ultimately results in recrudescence. Overall recurrence rates for colonic tumors are about 33% and for rectal cancer about 42%. Of these recurrences, about 9% are local, about 13% are systemic metastatic disease, and the remaining 88% are a combination of local and systemic disease. Fifty percent of patients with recurrent colorectal cancer have hepatic metastases.

Early detection of primary, metastatic, and recurrent disease can significantly impact the prognosis of individuals suffering from colorectal cancer. Large bowel cancer diagnosed at an early stage has a significantly better outcome than that diagnosed at more advanced stages. The 5 year relative survival rates for patients with regional or distant metastases are 48% and 5%, compared with 90% and 77% for disease which is *in situ* or local, respectively, at the time of diagnosis. Similarly, diagnosis of metastatic or recurrent disease earlier potentially carries with it a better prognosis.

Although current radiotherapeutic agents, chemotherapeutic agents and biological toxins are potent cytotoxins, they do not discriminate between normal and malignant cells, producing adverse effects and dose-limiting toxicities. Over the past decade, a novel approach has been employed to more specifically target agents to tumor cells, involving the conjugation of an active agent to molecules which binds preferentially to antigens that exist predominantly on tumor cells. These conjugates can be administered systemically and specifically bind to the targeted tumor cells. Theoretically, targeting permits uptake by cells of cytotoxic

agents at concentrations which do not produce serious toxicities in normal tissues. Also, selective binding to targeted tumor cells facilitates detection of occult tumor and is therefore useful in designing imaging agents. Molecular  
5 targeting predominantly has employed monoclonal antibodies generated to antigens selectively expressed on tumor cells.

Immunoscintigraphy using monoclonal antibodies directed at tumor-specific markers has been employed to diagnose colorectal cancer. Monoclonal antibodies against  
10 carcinoembryonic antigen (CEA) labeled with <sup>99</sup>Techneium identified 94% of patients with recurrent tumors. Similarly, <sup>111</sup>Indium-labeled anti-CEA monoclonal antibodies successfully diagnosed 85% of patients with recurrent colorectal carcinoma who were not diagnosed by conventional techniques. <sup>125</sup>Iodine-  
15 labeled antibodies have been effective in localizing more than 80% of the pathologically-confirmed recurrences by intraoperative gamma probe scanning.

Monoclonal antibodies have also been employed to target specific therapeutic agents in colorectal cancer.  
20 Preclinical studies demonstrated that anti-CEA antibodies labeled with <sup>90</sup>Yttrium inhibited human colon carcinoma xenografts in nude mice. Antibodies generated to colorectal cancer cells and coupled to mitomycin C or neocarzinostatin demonstrated an anti-tumor effect on human colon cancer  
25 xenografts in nude mice and 3 patients with colon cancer. Similar results in animals were obtained with monoclonal antibodies conjugated to ricin toxin A chain.

Due to the sensitivity, specificity, and adverse-effect profile of monoclonal antibodies, the results obtained  
30 using monoclonal antibody-based therapeutics have shown them to be less than ideal targeting tools. Although monoclonal antibodies have been generated to antigens selectively expressed on tumors, no truly cancer-specific antibody has been identified. Most antigens expressed on neoplastic cells appear  
35 to be quantitatively increased in these compared to normal cells but the antigens are nonetheless often present in normal cells. Thus, antibodies to such determinants can react with

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non-neoplastic tissues, resulting in significant toxicities. Also, antibodies are relatively large molecules and consequently, often evoke an immune response in patients. These immune responses can result in significant toxicities in  
5 patients upon re-exposure to the targeting agents and can prevent targeting by the monoclonal due to immune complex formation with degradation and excretion. Finally, binding of antibodies to tumor cells may be low and targeted agents may be delivered to cells in quantities insufficient to achieve  
10 detection or cytotoxicity.

There remains a need for compositions which can specifically target metastasized colorectal cancer cells. There is a need for imaging agents which can specifically bind  
15 to metastasized colorectal cancer cells. There is a need for improved methods of imaging metastasized colorectal cancer cells. There is a need for therapeutic agents which can specifically bind to metastasized colorectal cancer cells. There is a need for improved methods of treating individuals who are suspected of suffering from colorectal cancer cells,  
20 especially individuals who are suspected of suffering from metastasis of colorectal cancer cells.

#### SUMMARY OF THE INVENTION

The present invention relates to conjugated compounds which comprises an ST receptor binding moiety and a radiostable  
25 active moiety.

The present invention relates to a pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent, and a conjugated compound which comprises an ST receptor binding moiety and a radiostable active moiety.

30 The present invention relates to a method of treating an individual suspected of suffering from metastasized colorectal cancer comprising the steps of administering to said individual a pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent, and a  
35 therapeutically effective amount of a conjugated compound which

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comprises an ST receptor binding moiety and a radiostable active moiety.

The present invention relates to a pharmaceutical composition comprising a pharmaceutically acceptable carrier or  
5 diluent, and conjugated compound that comprises an ST receptor binding moiety and a radioactive active moiety wherein the conjugated compound is present in an amount effective for therapeutic or diagnostic use in humans suffering from colorectal cancer.

10 The present invention relates to a method of radioimaging metastasized colorectal cancer cells comprising the steps of first administering to an individual suspected of having metastasized colorectal cancer cells, a pharmaceutical composition that comprises a pharmaceutically acceptable  
15 carrier or diluent, and conjugated compound that comprises an ST receptor binding moiety and a radioactive active moiety wherein the conjugated compound is present in an amount effective for diagnostic use in humans suffering from colorectal cancer and then detecting the localization and  
20 accumulation of radioactivity in the individual's body.

The present invention relates to a method of treating an individual suspected of suffering from metastasized colorectal cancer comprising the steps of administering to said individual a pharmaceutical composition comprising a  
25 pharmaceutically acceptable carrier or diluent, and a therapeutically effective amount of a conjugated compound which comprises an ST receptor binding moiety and a radioactive active moiety.

#### DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTION

30 As used herein, the terms "ST" and "native ST" are used interchangeably and are meant to refer to heat-stable toxin (ST) which is a peptide produced by *E. coli*, as well as other organisms. STs are naturally occurring peptides which 1) are naturally produced by organisms, 2) which bind to the ST  
35 receptor and 3) which activate the signal cascade that mediates ST-induced diarrhea.

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As used herein, the term "radiostable" is meant to refer to compounds which do not undergo radioactive decay; i.e. compounds which are not radioactive.

As used herein, the term "therapeutic agent" is meant to refer to chemotherapeutics, toxins, radiotherapeutics, targeting agents or radiosensitizing agents.

As used herein, the term "chemotherapeutic" is meant to refer to compounds that, when contacted with and/or incorporated into a cell, produce an effect on the cell including causing the death of the cell, inhibiting cell division or inducing differentiation.

As used herein, the term "toxin" is meant to refer to compounds that, when contacted with and/or incorporated into a cell, produce the death of the cell.

As used herein, the term "radiotherapeutic" is meant to refer to radionuclides which when contacted with and/or incorporated into a cell, produce the death of the cell.

As used herein, the term "targeting agent" is meant to refer compounds which can be bound by and or react with other compounds. Targeting agents may be used to deliver chemotherapeutics, toxins, enzymes, radiotherapeutics, antibodies or imaging agents to cells that have targeting agents associated with them and/or to convert or otherwise transform or enhance coadministered active agents. A targeting agent may include a moiety that constitutes a first agent that is localized to the cell which when contacted with a second agent either is converted to a third agent which has a desired activity or causes the conversion of the second agent into an agent with a desired activity. The result is the localized agent facilitates exposure of an agent with a desired activity to the metastasized cell.

As used herein, the term "radiosensitizing agent" is meant to refer to agents which increase the susceptibility of cells to the damaging effects of ionizing radiation. A radiosensitizing agent permits lower doses of radiation to be administered and still provide a therapeutically effective dose.

As used herein, the term "imaging agent" is meant to refer to compounds which can be detected.

As used herein, the term "ST receptor binding moiety" is meant to refer to the portion of a conjugated compound that constitutes an ST receptor ligand.

As used herein, the term "active moiety" is meant to refer to the portion of a conjugated compound that constitutes an active agent.

As used herein, the terms "conjugated compound" and "conjugated composition" are used interchangeably and meant to refer to a compound which comprises an ST receptor binding moiety and an active moiety and which is capable of binding to the ST receptor. Conjugated compounds according to the present invention comprise a portion which constitutes an ST receptor ligand and a portion which constitutes an active agent. Thus, conjugated compounds according to the present invention are capable of specifically binding to the ST receptor and include a portion which is a therapeutic agent or imaging agent. Conjugated compositions may comprise crosslinkers and/or molecules that serve as spacers between the moieties.

As used herein, the terms "crosslinker", "crosslinking agent", "conjugating agent", "coupling agent", "condensation reagent" and "bifunctional crosslinker" are used interchangeably and are meant to refer to molecular groups which are used to attach the ST receptor ligand and the active agent to thus form the conjugated compound.

As used herein, the term "colorectal cancer" is meant to include the well-accepted medical definition that defines colorectal cancer as a medical condition characterized by cancer of cells of the intestinal tract below the small intestine (i.e. the large intestine (colon), including the cecum, ascending colon, transverse colon, descending colon, and sigmoid colon, and rectum). Additionally, as used herein, the term "colorectal cancer" is meant to further include medical conditions which are characterized by cancer of cells of the duodenum and small intestine (jejunum and ileum). The definition of colorectal cancer used herein is more expansive

As used herein, the term "metastasis" is meant to refer to the process in which cancer cells originating in one organ or part of the body relocate to another part of the body and continue to replicate. Metastasized cells subsequently form tumors which may further metastasize. Metastasis thus refers to the spread of cancer from the part of the body where it originally occurs to other parts of the body. The present invention relates to methods of delivering active agents to metastasized colorectal cancer cells.

ST, which is produced by *E. coli*, as well as other organisms, is responsible for endemic diarrhea in developing countries and travelers diarrhea. ST induces intestinal secretion by binding to specific receptors, ST receptors, in the apical brush border membranes of the mucosal cells lining the intestinal tract. Binding of ST to ST receptors is non-covalent and occurs in a concentration-dependent and saturable fashion. Once bound, ST-ST receptor complexes appear to be internalized by intestinal cells, i.e. transported from the surface into the interior of the cell. Binding of ST to ST receptors triggers a cascade of biochemical reactions in the apical membrane of these cells resulting in the production of a signal which induces intestinal cells to secrete fluids and electrolytes, resulting in diarrhea.

ST receptors are unique in that they are only localized in the apical brush border membranes of the cells

lining the intestinal tract. Indeed, they are not found in any other cell type in placental mammals. In addition, ST receptors are almost exclusively localized to the apical membranes, with little being found in the basolateral membranes on the sides of intestinal cells.

Mucosal cells lining the intestine are joined together by tight junctions which form a barrier against the passage of intestinal contents into the blood stream and components of the blood stream into the intestinal lumen. Therefore, the apical location of ST receptors isolates these receptors from the circulatory system so that they may be considered to exist separate from the rest of the body; essentially the "outside" of the body. Therefore, the rest of the body is considered "outside" the intestinal tract. Compositions administered "outside" the intestinal tract are maintained apart and segregated from the only cells which normally express ST receptors.

In individuals suffering from colorectal cancer, the cancer cells are often derived from cells that produce and display the ST receptor and these cancer cells continue to produce and display the ST receptor on their cell surfaces. Indeed, T84 cells, which are human colonic adenocarcinoma cells isolated from lung metastases, express ST receptors on their cell surface. Similarly, HT29glu-cells, which are human colonic adenocarcinoma cells, express receptors for ST. Thus, in individuals suffering from colorectal cancer, some metastasized intestinal cancer cells express ST receptors.

An effort was undertaken to determine the proportion of colorectal tumors which have the ST receptor. Sixteen colorectal cancer tumors, including ten local colorectal tumors and six metastasized tumors (3 liver, 1 lung, 1 lymphnode, 1 peritoneum), were tested and each possessed ST receptors. In each case, the affinity and density of receptors was amenable for targeting. That is, the cells possessed at least  $10^4$  -  $10^6$  receptors per cell and demonstrated an affinity of  $10^{-7}$  or better (that is preferably between  $10^{-8}$  to  $10^{-9}$  or less; the lower number indicating a tighter bond, thus a higher

affinity). Normal liver, lymphnode, peritoneum and lung cells were found not to possess ST receptors.

When such cancer cells metastasize, the metastasized cancer cells continue to produce and display the ST receptor.

5 The expression of ST receptors on the surfaces of metastatic tumors provides a target for selective binding of conjugated compositions. ST receptors permit the absolutely specific targeting of therapeutic and diagnostic agents that are conjugated to ST receptor ligands to metastatic colorectal  
10 cancer cells.

The conjugated compositions of the present invention are useful for targeting cells that line the inner intestine wall including those cancer cells derived from such cells, particularly metastasized cancer cells derived from such cells.

15 The conjugated compositions of the present invention which are administered outside the intestinal tract such as those administered in the circulatory system will remain segregated from the cells that line the intestinal tract and will bind only to cells outside the intestinal tract which are derived  
20 from the intestinal tract such as metastasized colorectal cells. The conjugated compositions will not bind to non-colorectal derived cells. Thus, the active moieties of conjugated compositions administered outside the intestinal tract are delivered to cells which are derived from the  
25 intestinal tract such as metastasized colorectal cells but will not be delivered to any other cells.

Therapeutic and diagnostic pharmaceutical compositions of the present invention include conjugated compounds specifically targeted to metastatic disease. These conjugated compounds include ST receptor binding moieties which do not bind to cells of normal tissue in the body except cells of the intestinal tract since the cells of other tissues do not possess ST receptors. Unlike normal colorectal cells and localized colorectal cancer cells, metastasized colorectal cancer cells are accessible to substances administered outside the intestinal tract, for example administered in the circulatory system. The only ST receptors in normal tissue

exist in the apical membranes of intestinal mucosa cells and these receptors are effectively isolated from the targeted cancer chemotherapeutics and imaging agents administered outside the intestinal tract by the intestinal mucosa barrier.

5 Thus, metastasized colorectal cells may be targeted by conjugated compounds of the present invention by introducing such compounds outside the intestinal tract such as for example by administering pharmaceutical compositions that comprise conjugated compounds into the circulatory system.

10 One having ordinary skill in the art can readily identify individuals suspected of suffering from colorectal cancer and metastasized colorectal cells. In those individuals diagnosed with colorectal cancer, it is standard therapy to suspect metastasis and aggressively attempt to eradicate  
15 metastasized cells. The present invention provides pharmaceutical compositions and methods for imaging and thereby will more definitively diagnose metastasis. Further, the present invention provides pharmaceutical compositions comprising therapeutic agents and methods for specifically  
20 targeting and eliminating metastasized colorectal cancer cells. Further, the present invention provides pharmaceutical compositions that comprise therapeutics and methods for specifically eliminating colorectal cancer cells.

The pharmaceutical compositions which comprise  
25 conjugated compositions of the present invention may be used to diagnose or treat individuals suffering from localized colorectal tumors, that is primary or non-metastatic colorectal tumors if these have penetrated the basement membrane underlying the mucosa into the submucosa where there is  
30 abundant blood supply to which they have access. Penetration into the submucosa circumvents the mucosal barrier resulting in the ability of conjugated compositions introduced into the circulatory system to interact with these tumors.

The present invention relies upon the use of an ST  
35 receptor binding moiety in a conjugated composition. The ST receptor binding moiety is essentially a portion of the conjugated composition which acts as a ligand to the ST

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receptor and thus specifically binds to these receptors. The conjugated composition also includes an active moiety which is associated with the ST receptor binding moiety; the active moiety being an active agent which is either useful to image, target, neutralize or kill the cell.

According to the present invention, the ST receptor binding moiety is the ST receptor ligand portion of a conjugated composition. In some embodiments, the ST receptor ligand may be native ST.

Native ST has been isolated from a variety of organisms including *E. coli*, *Yersinia*, *Enterobacter*, and others. In nature, the toxins are generally encoded on a plasmid which can "jump" between different species. Several different toxins have been reported to occur in different species. These toxins all possess significant sequence homology, they all bind to ST receptors and they all activate guanylate cyclase, producing diarrhea.

ST has been both cloned and synthesized by chemical techniques. The cloned or synthetic molecules exhibit binding characteristics which are similar to native ST. Native ST isolated from *E. coli* is 18 or 19 amino acids in length. The smallest "fragment" of ST which retains activity is the 13 amino acid core peptide extending toward the carboxy terminal from cysteine 6 to cysteine 18 (of the 19 amino acid form). Analogues of ST have been generated by cloning and by chemical techniques. Small peptide fragments of the native ST structure which include the structural determinant that confers binding activity may be constructed. Once a structure is identified which binds to ST receptors, non-peptide analogues mimicking that structure in space are designed.

SEQ ID NO:1 discloses a nucleotide sequence which encodes 19 amino acid ST, designated ST Ia, reported by So and McCarthy (1980) *Proc. Natl. Acad. Sci. USA* 77:4011, which is incorporated herein by reference.

The amino acid sequence of ST Ia is disclosed in SEQ ID NO:2.

SEQ ID NO:3 discloses the amino acid sequence of an 18 amino acid peptide which exhibits ST activity, designated ST I\*, reported by Chan and Giannella (1981) *J. Biol. Chem.* 256:7744, which is incorporated herein by reference.

5           SEQ ID NO:4 discloses a nucleotide sequence which  
encodes 19 amino acid ST, designated ST Ib, reported by Mosely  
et al. (1983) *Infect. Immun.* **39**:1167, which is incorporated  
herein by reference.

The amino acid sequence of ST Ib is disclosed in SEQ  
10 ID NO:5.

A 15 amino acid peptide called guanylin which has about 50% sequence homology to ST has been identified in mammalian intestine (Currie, M.G. et al. (1992) *Proc. Natl. Acad. Sci. USA* **89**:947-951, which is incorporated herein by reference). Guanylin binds to ST receptors and activates guanylate cyclase at a level of about 10- to 100-fold less than native ST. Guanylin may not exist as a 15 amino acid peptide in the intestine but rather as part of a larger protein in that organ. The amino acid sequence of guanylin from rodent is disclosed as SEQ ID NO:6.

SEQ ID NO:7 is an 18 amino acid fragment of SEQ ID NO:2. SEQ ID NO:8 is a 17 amino acid fragment of SEQ ID NO:2. SEQ ID NO:9 is a 16 amino acid fragment of SEQ ID NO:2. SEQ ID NO:10 is a 15 amino acid fragment of SEQ ID NO:2. SEQ ID NO:11 is a 14 amino acid fragment of SEQ ID NO:2. SEQ ID NO:12 is a 13 amino acid fragment of SEQ ID NO:2. SEQ ID NO:13 is an 18 amino acid fragment of SEQ ID NO:2. SEQ ID NO:14 is a 17 amino acid fragment of SEQ ID NO:2. SEQ ID NO:15 is a 16 amino acid fragment of SEQ ID NO:2. SEQ ID NO:16 is a 15 amino acid fragment of SEQ ID NO:2. SEQ ID NO:17 is a 14 amino acid fragment of SEQ ID NO:2.

SEQ ID NO:18 is a 17 amino acid fragment of SEQ ID  
NO:3. SEQ ID NO:19 is a 16 amino acid fragment of SEQ ID NO:3.  
SEQ ID NO:20 is a 15 amino acid fragment of SEQ ID NO:3. SEQ  
ID NO:21 is a 14 amino acid fragment of SEQ ID NO:3. SEQ ID  
NO:22 is a 13 amino acid fragment of SEQ ID NO:3. SEQ ID NO:23  
is a 17 amino acid fragment of SEQ ID NO:3. SEQ ID NO:24 is a



16 amino acid fragment of SEQ ID NO:3. SEQ ID NO:25 is a 15 amino acid fragment of SEQ ID NO:3. SEQ ID NO:26 is a 14 amino acid fragment of SEQ ID NO:3.

SEQ ID NO:27 is an 18 amino acid fragment of SEQ ID NO:5. SEQ ID NO:28 is a 17 amino acid fragment of SEQ ID NO:5. SEQ ID NO:29 is a 16 amino acid fragment of SEQ ID NO:5. SEQ ID NO:30 is a 15 amino acid fragment of SEQ ID NO:5. SEQ ID NO:31 is a 14 amino acid fragment of SEQ ID NO:5. SEQ ID NO:32 is a 13 amino acid fragment of SEQ ID NO:5. SEQ ID NO:33 is an 18 amino acid fragment of SEQ ID NO:5. SEQ ID NO:34 is a 17 amino acid fragment of SEQ ID NO:5. SEQ ID NO:35 is a 16 amino acid fragment of SEQ ID NO:5. SEQ ID NO:36 is a 15 amino acid fragment of SEQ ID NO:5. SEQ ID NO:37 is a 14 amino acid fragment of SEQ ID NO:5.

SEQ ID NO:27, SEQ ID NO:31, SEQ ID NO:36 AND SEQ ID NO:37 are disclosed in Yoshimura, S., et al. (1985) *FEBS Lett.* **181**:138, which is incorporated herein by reference.

SEQ ID NO:38, SEQ ID NO:39 and SEQ ID NO:40, which are derivatives of SEQ ID NO:3, are disclosed in Waldman, S.A. and O'Hanley, P. (1989) *Infect. Immun.* **57**:2420, which is incorporated herein by reference.

SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44 and SEQ ID NO:45, which are a derivatives of SEQ ID NO:3, are disclosed in Yoshimura, S., et al. (1985) *FEBS Lett.* **181**:138, which is incorporated herein by reference.

SEQ ID NO:46 is a 25 amino acid peptide derived from *Y. enterocolitica* which binds to the ST receptor.

SEQ ID NO:47 is a 16 amino acid peptide derived from *V. cholerae* which binds to the ST receptor. SEQ ID NO:47 is reported in Shimonishi, Y., et al. *FEBS Lett.* **215**:165, which is incorporated herein by reference.

SEQ ID NO:48 is an 18 amino acid peptide derived from *Y. enterocolitica* which binds to the ST receptor. SEQ ID NO:48 is reported in Okamoto, K., et al. *Infec. Immun.* **55**:2121, which is incorporated herein by reference.

SEQ ID NO:49, is a derivative of SEQ ID NO:5.

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The diagram illustrates the experimental setup. A participant is seated at a table, looking at a video screen. On the screen, a target (a small circle) is visible. The participant's hand is positioned at a starting point (a larger circle). The distance between the starting point and the target is labeled 'Distance'. The participant's hand is labeled 'Hand'. The video screen is labeled 'Video screen'. The participant is labeled 'Participant'.

The diagram illustrates the experimental setup. A participant is seated at a table, looking at a video screen. On the screen, a target (a small circle) is visible. The participant's hand is positioned at a starting point (a larger circle). The distance between the starting point and the target is labeled 'Distance'. The participant's hand is labeled 'Hand'. The video screen is labeled 'Video screen'. The participant is labeled 'Participant'.

The diagram illustrates the experimental setup. A participant is seated at a table, looking at a video screen. On the screen, a target (a small circle) is visible. The participant's hand is positioned at a starting point (a larger circle). The distance between the starting point and the target is labeled 'Distance'. The participant's hand is labeled 'Hand'. The video screen is labeled 'Video screen'. The participant is labeled 'Participant'.

The figure consists of seven subplots arranged vertically, each representing a different iteration \$k\$ from 0 to 7. Each subplot shows a histogram of the number of non-zero elements in the vector \$\mathbf{x}\_k\$. The x-axis for all plots ranges from 0 to 200. The y-axis represents frequency, with scales varying between plots (e.g., 0-10 for \$k=0\$, 0-8 for \$k=1\$, etc.). The distributions are roughly bell-shaped and centered around 100-150 non-zero elements.

[illegible][illegible]

may comprise additional amino acid residues required to achieve proper three dimensional conformation including residues which facilitate circularization or desired folding.

It is preferred that the ST receptor ligand used as the ST receptor binding moiety be as small as possible. Thus it is preferred that the ST receptor ligand be a non-peptide small molecule or small peptide, preferably less than 25 amino acids, more preferably less than 20 amino acids. In some embodiments, the ST receptor ligand which constitute the ST receptor binding moiety of a conjugated composition is less than 15 amino acids. ST receptor binding peptide comprising less than 10 amino acids and ST receptor binding peptide less than 5 amino acids may be used as ST binding moieties according to the present invention. It is within the scope of the present invention to include larger molecules which serve as ST receptor binding moieties including, but not limited to molecules such as antibodies, FAbs and F(Ab)<sub>2</sub>s which specifically bind to ST receptor.

An assay may be used to test both peptide and non-peptide compositions to determine whether or not they are ST receptor ligands or, to test conjugated compositions to determine if they possess ST receptor binding activity. Such compositions that specifically bind to ST receptors can be identified by a competitive binding assay. The competitive binding assay is a standard technique in pharmacology which can be readily performed by those having ordinary skill in the art using readily available starting materials. Competitive binding assays have been shown to be effective for identifying compositions that specifically bind to ST receptors. Briefly, the assay consists of incubating a preparation of ST receptors (e.g. intestinal membranes from rat intestine, human intestine, T84 cells) with a constant concentration ( $1 \times 10^{-10}$  M to  $5 \times 10^{-10}$  M) of <sup>125</sup>I-ST (any ST receptor ligand such as native STs SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:5 may be used) and a known concentration of a test compound. As a control, a duplicate preparation of ST receptors are incubated with a duplicate concentration of <sup>125</sup>I-ST in the absence of test compound.

Assays are incubated to equilibrium (2 hours) and the amount of  $^{125}\text{I}$ -ST bound to receptors is quantified by standard techniques. The ability of the test compound to bind to receptors is measured as its ability to prevent (compete with) the  $^{125}\text{I}$ -ST from binding. Thus, in assays containing the test compound which bind to the receptor, there will be less radioactivity associated with the receptors. This assay, which is appropriate for determining the ability of any molecule to bind to ST receptors, is a standard competitive binding assay which can be readily employed by those having ordinary skill in the art using readily available starting materials.

ST may be isolated from natural sources using standard techniques. Additionally, ST receptor binding peptides and conjugated compositions or portions thereof which are peptides may be prepared routinely by any of the following known techniques.

ST receptor binding peptides and conjugated compositions or portions thereof which are peptides may be prepared using the solid-phase synthetic technique initially described by Merrifield, in *J. Am. Chem. Soc.*, 15:2149-2154 (1963). Other peptide synthesis techniques may be found, for example, in M. Bodanszky et al., (1976) *Peptide Synthesis*, John Wiley & Sons, 2d Ed.; Kent and Clark-Lewis in *Synthetic Peptides in Biology and Medicine*, p. 295-358, eds. Alitalo, K., et al. Science Publishers, (Amsterdam, 1985); as well as other reference works known to those skilled in the art. A summary of peptide synthesis techniques may be found in J. Stuart and J.D. Young, *Solid Phase Peptide Synthesis*, Pierce Chemical Company, Rockford, IL (1984), which is incorporated herein by reference. The synthesis of peptides by solution methods may also be used, as described in *The Proteins*, Vol. II, 3d Ed., p. 105-237, Neurath, H. et al., Eds., Academic Press, New York, NY (1976). Appropriate protective groups for use in such syntheses will be found in the above texts, as well as in J.F.W. McOmie, *Protective Groups in Organic Chemistry*, Plenum Press, New York, NY (1973), which is incorporated herein by reference. In general, these synthetic methods involve the

sequential addition of one or more amino acid residues or suitable protected amino acid residues to a growing peptide chain. Normally, either the amino or carboxyl group of the first amino acid residue is protected by a suitable, selectively removable protecting group. A different, selectively removable protecting group is utilized for amino acids containing a reactive side group, such as lysine.

Using a solid phase synthesis as an example, the protected or derivatized amino acid is attached to an inert solid support through its unprotected carboxyl or amino group. The protecting group of the amino or carboxyl group is then selectively removed and the next amino acid in the sequence having the complementary (amino or carboxyl) group suitably protected is admixed and reacted with the residue already attached to the solid support. The protecting group of the amino or carboxyl group is then removed from this newly added amino acid residue, and the next amino acid (suitably protected) is then added, and so forth. After all the desired amino acids have been linked in the proper sequence, any remaining terminal and side group protecting groups (and solid support) are removed sequentially or concurrently, to provide the final peptide. The peptide of the invention are preferably devoid of benzylated or methylbenzylated amino acids. Such protecting group moieties may be used in the course of synthesis, but they are removed before the peptides are used. Additional reactions may be necessary, as described elsewhere, to form intramolecular linkages to restrain conformation.

ST receptor binding peptides and conjugated compositions or portions thereof which are peptides may also be prepared by recombinant DNA techniques. Provision of a suitable DNA sequence encoding the desired peptide permits the production of the peptide using recombinant techniques now known in the art. The coding sequence can be obtained from natural sources or synthesized or otherwise constructed using widely available starting materials by routine methods. When the coding DNA is prepared synthetically, advantage can be

taken of known codon preferences of the intended host where the DNA is to be expressed.

To produce an ST receptor binding peptide which occurs in nature, one having ordinary skill in the art can, using well-known techniques, obtain a DNA molecule encoding the ST receptor binding peptides from the genome of the organism that produces the ST receptor binding peptide and insert that DNA molecule into a commercially available expression vector for use in well-known expression systems.

Likewise, one having ordinary skill in the art can, using well known techniques, combine a DNA molecule that encodes an ST receptor binding peptide, such as SEQ ID NO:1 and SEQ ID NO:4, which can be obtained from the genome of the organism that produces the ST, with DNA that encodes a toxin, another active agent that is a peptide or additionally, any other amino acid sequences desired to be adjacent to the ST receptor binding peptide amino acid sequence in a single peptide and insert that DNA molecule into a commercially available expression vector for use in well-known expression systems.

For example, the commercially available plasmid pSE420 (Invitrogen, San Diego, CA) may be used for recombinant production in *E. coli*. The commercially available plasmid pYES2 (Invitrogen, San Diego, CA) may be used for production in *S. cerevisiae* strains of yeast. The commercially available MaxBac™ (Invitrogen, San Diego, CA) complete baculovirus expression system may be used for production in insect cells. The commercially available plasmid pcDNA I (Invitrogen, San Diego, CA) may be used for production in mammalian cells such as Chinese Hamster Ovary cells.

One having ordinary skill in the art may use these or other commercially available expression vectors and systems or produce vectors using well-known methods and readily available starting materials. Expression systems containing the requisite control sequences, such as promoters and polyadenylation signals, and preferably enhancers, are readily available and known in the art for a variety of hosts. See

e.g., Sambrook et al., *Molecular Cloning a Laboratory Manual*, Second Ed. Cold Spring Harbor Press (1989). Thus, the desired proteins can be prepared in both prokaryotic and eukaryotic systems, resulting in a spectrum of processed forms of the  
5 protein.

The most commonly used prokaryotic system remains *E. coli*, although other systems such as *B. subtilis* and *Pseudomonas* are also useful. Suitable control sequences for prokaryotic systems include both constitutive and inducible promoters including the *lac* promoter, the *trp* promoter, hybrid promoters such as *tac* promoter, the *lambda* phage P1 promoter. In general, foreign proteins may be produced in these hosts either as fusion or mature proteins. When the desired sequences are produced as mature proteins, the sequence produced may be preceded by a methionine which is not necessarily efficiently removed. Accordingly, the peptides and proteins claimed herein may be preceded by an N-terminal Met when produced in bacteria. Moreover, constructs may be made wherein the coding sequence for the peptide is preceded by an operable signal peptide which results in the secretion of the protein. When produced in prokaryotic hosts in this matter, the signal sequence is removed upon secretion.

A wide variety of eukaryotic hosts are also now available for production of recombinant foreign proteins. As in bacteria, eukaryotic hosts may be transformed with expression systems which produce the desired protein directly, but more commonly signal sequences are provided to effect the secretion of the protein. Eukaryotic systems have the additional advantage that they are able to process introns which may occur in the genomic sequences encoding proteins of higher organisms. Eukaryotic systems also provide a variety of processing mechanisms which result in, for example, glycosylation, carboxy-terminal amidation, oxidation or derivatization of certain amino acid residues, conformational control, and so forth.

Commonly used eukaryotic systems include, but are not limited to, yeast, fungal cells, insect cells, mammalian cells,

avian cells, and cells of higher plants. Suitable promoters are available which are compatible and operable for use in each of these host types as well as are termination sequences and enhancers, as e.g. the baculovirus polyhedron promoter. As  
5 above, promoters can be either constitutive or inducible. For example, in mammalian systems, the mouse metallothione promoter can be induced by the addition of heavy metal ions.

The particulars for the construction of expression systems suitable for desired hosts are known to those in the  
10 art. For recombinant production of the protein, the DNA encoding it is suitably ligated into the expression vector of choice and then used to transform the compatible host which is then cultured and maintained under conditions wherein expression of the foreign gene takes place. The protein of the  
15 present invention thus produced is recovered from the culture, either by lysing the cells or from the culture medium as appropriate and known to those in the art.

One having ordinary skill in the art can, using well-known techniques, isolate the protein that is produced.

20 According to the present invention, the active moiety may be a therapeutic agent or an imaging agent. One having ordinary skill in the art can readily recognize the advantages of being able to specifically target metastasized colorectal cells with an ST receptor ligand and conjugate such a ligand  
25 with many different active agents.

Chemotherapeutics useful as active moieties which when conjugated to an ST receptor binding moiety are specifically delivered to metastasized colorectal cells are typically, small chemical entities produced by chemical synthesis.  
30 Chemotherapeutics include cytotoxic and cytostatic drugs. Chemotherapeutics may include those which have other effects on cells such as reversal of the transformed state to a differentiated state or those which inhibit cell replication. Examples of chemotherapeutics include common cytotoxic or  
35 cytostatic drugs such as for example: methotrexate (amethopterin), doxorubicin (adrimycin), daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan,



chlorambucil, and other nitrogen mustards (e.g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: purothionin (barley flour oligopeptide), macromomycin.

5 1,4-benzoquinone derivatives and trenimon.

Toxins are useful as active moieties. When a toxin is conjugated to an ST receptor binding moiety, the conjugated composition is specifically delivered to a metastasized colorectal cell by way of the ST receptor binding moiety and the toxin moiety kills the cell. Toxins are generally complex  
10 toxic products of various organisms including bacteria, plants, etc. Examples of toxins include but are not limited to: ricin, ricin A chain (ricin toxin), *Pseudomonas* exotoxin (PE), diphtheria toxin (DT), *Clostridium perfringens* phospholipase C  
15 (PLC), bovine pancreatic ribonuclease (BPR), pokeweed antiviral protein (PAP), abrin, abrin A chain (abrin toxin), cobra venom factor (CVF), gelonin (GEL), saporin (SAP), modeccin, viscumin and volkensin. As discussed above, when protein toxins are employed with ST receptor binding peptides, conjugated  
20 compositions may be produced using recombinant DNA techniques. Briefly, a recombinant DNA molecule can be constructed which encodes both the ST receptor ligand and the toxin on a chimeric gene. When the chimeric gene is expressed, a fusion protein is produced which includes an ST receptor binding moiety and an  
25 active moiety. Protein toxins are also useful to form conjugated compounds with ST receptor binding peptides through non-peptidyl bonds.

In addition, there are other approaches for utilizing active agents for the treatment of cancer. For example,  
30 conjugated compositions may be produced which include an ST binding moiety and an active moiety which is an active enzyme. The ST binding moiety specifically localizes the conjugated composition to the tumor cells. An inactive prodrug which can be converted by the enzyme into an active drug is administered  
35 to the patient. The prodrug is only converted to an active drug by the enzyme which is localized to the tumor. An example of an enzyme/prodrug pair includes alkaline phosphatase/



imaging (MRI) or computed tomography (CT scan). The most commonly employed radionuclide imaging agents include radioactive iodine and indium. Imaging by CT scan may employ a heavy metal such as iron chelates. MRI scanning may employ  
5 chelates of gadolinium or manganese. Additionally, positron emission tomography (PET) may be possible using positron emitters of oxygen, nitrogen, iron, carbon, or gallium. Example of radionuclides useful in imaging procedures include:  
10 <sup>43</sup>K, <sup>52</sup>Fe, <sup>57</sup>Co, <sup>67</sup>Cu, <sup>67</sup>Ga, <sup>68</sup>Ga, <sup>77</sup>Br, <sup>81</sup>Rb/<sup>81m</sup>Kr, <sup>87m</sup>Sr, <sup>99m</sup>Tc, <sup>111</sup>In, <sup>113m</sup>In, <sup>123</sup>I, <sup>125</sup>I, <sup>127</sup>Cs, <sup>129</sup>Cs, <sup>131</sup>I, <sup>132</sup>I, <sup>197</sup>Hg, <sup>203</sup>Pb and <sup>206</sup>Bi.

It is preferred that the conjugated compositions be non-immunogenic or immunogenic at a very low level. Accordingly, it is preferred that the ST receptor binding moiety be a small, poorly immunogenic or non-immunogenic  
15 peptide or a non-peptide. Likewise, it is preferred that the active moiety be a small, poorly-immunogenic or non-immunogenic peptide or a non-peptide. Native ST, being a small peptide, has been shown to poorly immunogenic. (See: Klipstein, F.A. et al. (1982) *Infect. Immun.* **37**:550-557; Giannella, R.A. et al.  
20 (1981) *Infect. Immun.* **33**:186; Burgess, M.N. et al. (1978) *Infect. Immun.* **21**:60; Evans, D.G. et al. (1973) *Infect. Immun.* **7**:873; Gyles, C.L. (1979) *Ann. N.Y. Acad. Sci.* **16**:314; and Sack, R.B. (1975) *Ann. Rev. Microbiol.* **29**:333.) Similarly, fragments and amino acid substitute derivatives of native ST  
25 are poorly immunogenic. Accordingly, conjugated compositions which include all or part of the native ST as an ST receptor binding moiety are generally poorly immunogenic to the extent that the native ST is poorly immunogenic.

ST receptor ligands are conjugated to active agents  
30 by a variety of well-known techniques readily performed without undue experimentation by those having ordinary skill in the art. The technique used to conjugate the ST receptor ligand to the active agent is dependent upon the molecular nature of the ST receptor ligand and the active agent. After the ST receptor  
35 ligand and the active agent are conjugated to form a single molecule, assays may be performed to ensure that the conjugated molecule retains the activities of the moieties. The ST

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receptor binding assay described above may be performed using the conjugated compound to test whether it is capable of binding to the ST receptor. Similarly, the activity of the active moiety may be tested using various assays for each  
5 respective type of active agent. Radionuclides retain there activity, i.e. their radioactivity, irrespective of conjugation. With respect to active agents which are toxins, drugs and targeting agents, standard assays to demonstrate the activity of unconjugated forms of these compounds may be used  
10 to confirm that the activity has been retained.

Conjugation may be accomplished directly between the ST receptor ligand and the active agent or linking, intermediate molecular groups may be provided between the ST receptor ligand and the active agent. Crosslinkers are  
15 particularly useful to facilitate conjugation by providing attachment sites for each moiety. Crosslinkers may include additional molecular groups which serve as spacers to separate the moieties from each other to prevent either from interfering with the activity of the other.

20 In some preferred embodiments, the ST receptor ligand peptide is SEQ ID NO:2, SEQ ID NO:3, SEQ ID NOS:5-54 or fragments or derivatives thereof. It has been observed that conjugation to these molecules is preferably performed at the amino terminus of each respective peptide. In ST receptor  
25 ligand peptides comprising D amino acid sequences in the opposite order as SEQ ID NO:2, SEQ ID NO:3, SEQ ID NOS:5-54, conjugation preferably is performed at the carboxy terminus.

One having ordinary skill in the art may conjugate an ST receptor ligand peptide to a chemotherapeutic drug using  
30 well-known techniques. For example, Magerstadt, M. *Antibody Conjugates and Malignant Disease*. (1991) CRC Press, Boca Raton, USA, pp. 110-152) which is incorporated herein by reference, teaches the conjugation of various cytostatic drugs to amino acids of antibodies. Such reactions may be applied to  
35 conjugate chemotherapeutic drugs to ST receptor ligands, including ST receptor binding peptides, with an appropriate linker. ST receptor ligands which have a free amino group such

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as ST receptor binding peptides may be conjugated to active agents at that group. Most of the chemotherapeutic agents currently in use in treating cancer possess functional groups that are amenable to chemical crosslinking directly with proteins. For example, free amino groups are available on methotrexate, doxorubicin, daunorubicin, cytosinarabioside, *cis*-platin, vindesine, mitomycin and bleomycin while free carboxylic acid groups are available on methotrexate, melphalan, and chlorambucil. These functional groups, that is free amino and carboxylic acids, are targets for a variety of homobifunctional and heterobifunctional chemical crosslinking agents which can crosslink these drugs directly to the single free amino group of ST. For example, one procedure for crosslinking ST receptor ligands which have a free amino group such as ST receptor binding peptides, as for example SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5-54 to active agents which have a free amino group such as methotrexate, doxorubicin, daunorubicin, cytosinarabioside, *cis*-platin, vindesine, mitomycin and bleomycin, or alkaline phosphatase, or protein- or peptide-based toxin employs homobifunctional succinimidyl esters, preferably with carbon chain spacers such as disuccinimidyl suberate (Pierce Co, Rockford, IL). In the event that a cleavable conjugated compound is required, the same protocol would be employed utilizing 3,3'- dithiobis (sulfosuccinimidylpropionate; Pierce Co.).

In order to conjugate an ST receptor ligand peptide to a peptide-based active agent such as a toxin, the ST receptor ligand and the toxin may be produced as a single, fusion protein either by standard peptide synthesis or recombinant DNA technology, both of which can be routinely performed by those having ordinary skill in the art. Alternatively, two peptides, the ST receptor ligand peptide and the peptide-based toxin may be produced and/or isolated as separate peptides and conjugated using crosslinkers. As with conjugated compositions that contain chemotherapeutic drugs, conjugation of ST receptor binding peptides and toxins can exploit the ability to modify the single free amino group of an

ST receptor binding peptide while preserving the receptor-binding function of this molecule.

One having ordinary skill in the art may conjugate an ST receptor ligand peptide to a radionuclide using well-known techniques. For example, Magerstadt, M. (1991) *Antibody Conjugates And Malignant Disease*, CRC Press, Boca Raton, FLA,; and Barchel, S.W. and Rhodes, B.H., (1983) *Radioimaging and Radiotherapy*, Elsevier, NY, NY, each of which is incorporated herein by reference, teach the conjugation of various therapeutic and diagnostic radionuclides to amino acids of antibodies. Such reactions may be applied to conjugate radionuclides to ST receptor ligand peptides or to ST receptor ligands including ST receptor ligand peptides with an appropriate linker.

The present invention provides pharmaceutical compositions that comprise the conjugated compounds of the invention and pharmaceutically acceptable carriers or diluents. The pharmaceutical composition of the present invention may be formulated by one having ordinary skill in the art. Suitable pharmaceutical carriers are described in *Remington's Pharmaceutical Sciences*, A. Osol, a standard reference text in this field, which is incorporated herein by reference. In carrying out methods of the present invention, conjugated compounds of the present invention can be used alone or in combination with other diagnostic, therapeutic or additional agents. Such additional agents include excipients such as coloring, stabilizing agents, osmotic agents and antibacterial agents.

The conjugated compositions of the invention can be, for example, formulated as a solution, suspension or emulsion in association with a pharmaceutically acceptable parenteral vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Liposomes may also be used. The vehicle may contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by commonly used techniques. For

example, a parenteral composition suitable for administration by injection is prepared by dissolving 1.5% by weight of active ingredient in 0.9% sodium chloride solution.

The pharmaceutical compositions according to the present invention may be administered as either a single dose or in multiple doses. The pharmaceutical compositions of the present invention may be administered either as individual therapeutic agents or in combination with other therapeutic agents. The treatments of the present invention may be combined with conventional therapies, which may be administered sequentially or simultaneously.

The pharmaceutical compositions of the present invention may be administered by any means that enables the conjugated composition to reach the targeted cells. In some  
15 embodiments, routes of administration include those selected from the group consisting of intravenous, intraarterial, intraperitoneal, local administration into the blood supply of the organ in which the tumor resides or directly into the tumor itself. Intravenous administration is the preferred mode of  
20 administration. It may be accomplished with the aid of an infusion pump.

The dosage administered varies depending upon factors such as: the nature of the active moiety; the nature of the conjugated composition; pharmacodynamic characteristics; its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms; kind of concurrent treatment; and frequency of treatment.

Because conjugated compounds are specifically targeted to cells with ST receptors, conjugated compounds which comprise  
30 chemotherapeutics or toxins are administered in doses less than those which are used when the chemotherapeutics or toxins are administered as unconjugated active agents, preferably in doses that contain up to 100 times less active agent. In some  
embodiments, conjugated compounds which comprise  
35 chemotherapeutics or toxins are administered in doses that contain 10-100 times less active agent as an active moiety than the dosage of chemotherapeutics or toxins administered as

unconjugated active agents. To determine the appropriate dose, the amount of compound is preferably measured in moles instead of by weight. In that way, the variable weight of different ST binding moieties does not affect the calculation. Presuming a one to one ratio of ST binding moiety to active moiety in conjugated compositions of the invention, less moles of conjugated compounds may be administered as compared to the moles of unconjugated compounds administered, preferably up to 100 times less moles.

Typically, chemotherapeutic conjugates are administered intravenously in multiple divided doses.

Up to 20 gm IV/dose of methotrexate is typically administered in an unconjugated form. When methotrexate is administered as the active moiety in a conjugated compound of the invention, there is a 10-to 100-fold dose reduction. Thus, presuming each conjugated compound includes one molecule of methotrexate conjugated to one ST receptor binding moiety, of the total amount of conjugated compound administered, up to about 0.2 - 2.0 g of methotrexate is present and therefore administered. In some embodiments, of the total amount of conjugated compound administered, up to about 200 mg - 2g of methotrexate is present and therefore administered.

Methotrexate has a molecular weight of 455. One mole of the ST peptide-methotrexate conjugate weighs between about 1755-2955 depending on the ST peptide used. The effective dose range for ST peptide-methotrexate conjugate is between about 10 to 1000 mg. In some embodiments, dosages of 50 to 500 mg of ST peptide-methotrexate conjugate are administered. In some embodiments, dosages of 80 to 240 mg of ST peptide-methotrexate conjugate are administered.

Doxorubicin and daunorubicin each weigh about 535. Thus, ST peptide-doxorubicin conjugates and ST peptide-daunorubicin conjugates each have molecular weights of between about 1835-2553.5. Presuming each conjugated compound includes one molecule of doxorubicin or daunorubicin conjugated to one ST receptor binding moiety, the effective dose range for ST peptide-doxorubicin conjugate or ST peptide-daunorubicin



conjugate is between about 40 to 4000 mg. In some embodiments, dosages of 100 to 1000 mg of ST peptide-doxorubicin conjugate or ST peptide-daunorubicin conjugate are administered. In some  
5 conjugate or ST peptide-daunorubicin conjugate are administered.

Toxin-containing conjugated compounds are formulated for intravenous administration. Using this approach, up to 6  
10 nanomoles/kg of body weight of toxin have been administered as a single dose with marked therapeutic effects in patients with melanoma (Spitler L.E., et al. (1987) *Cancer Res.* 47:1717). In some embodiments, up to about 11 micrograms of ST peptide-toxin conjugated compound/kg of body weight may be administered for therapy.

15 Presuming each conjugated compound includes one molecule of ricin toxin A chain conjugated to an ST receptor binding moiety, conjugated compositions comprising ricin toxin A chain are administered in doses in which the proportion by weight of ricin toxin A chain is 1-500  $\mu$ g of the total weight  
20 of the conjugated compound administered. In some preferred embodiments, conjugated compositions comprising ricin toxin A chain are administered in doses in which the proportion by weight of ricin toxin A chain is 10-100  $\mu$ g of the total weight of the conjugated compound administered. In some preferred  
25 embodiments, conjugated compositions comprising ricin toxin A chain are administered in doses in which the proportion by weight of ricin toxin A chain is 2-50  $\mu$ g of the total weight of the conjugated compound administered. The molecular weight of ricin toxin A chain is 32,000. Thus, a conjugated compound  
30 that contains ricin A chain linked to an ST peptide has a molecular weight of about 33,300-34,500. The range of doses of such conjugated compounds to be administered are 1 to 500  $\mu$ g. In some embodiments, 10 to 100  $\mu$ g of such conjugated compounds are administered. In some embodiments, 20 to 50  $\mu$ g of such  
35 conjugated compounds are administered.

Presuming each conjugated compound includes one molecule of diphtheria toxin A chain conjugated to an ST

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receptor binding moiety, conjugated compositions comprising diphtheria toxin A chain are administered in doses in which the proportion by weight of diphtheria toxin A chain is 1-500  $\mu\text{g}$  of the total weight of the conjugated compound administered. In some preferred embodiments, conjugated compositions comprising diphtheria toxin A chain are administered in doses in which the proportion by weight of diphtheria toxin A chain is 10-100  $\mu\text{g}$  of the total weight of the conjugated compound administered. In some preferred embodiments, conjugated compositions comprising diphtheria toxin A chain are administered in doses in which the proportion by weight of diphtheria toxin A chain is 40-80  $\mu\text{g}$  of the total weight of the conjugated compound administered. The molecular weight of diphtheria toxin A chain is 66,600. Thus, a conjugated compound that contains diphtheria A chain linked to an ST peptide has a molecular weight of about 67,900-69,100. The range of doses of such conjugated compounds to be administered tested are 1 to 500  $\mu\text{g}$ . In some embodiments, 10 to 100  $\mu\text{g}$  of such conjugated compounds are administered. In some embodiments, 40 to 80  $\mu\text{g}$  of such conjugated compounds are administered.

Presuming each conjugated compound includes one molecule of *Pseudomonas* exotoxin conjugated to an ST receptor binding moiety, conjugated compositions comprising *Pseudomonas* exotoxin are administered in doses in which the proportion by weight of *Pseudomonas* exotoxin is .01 - 100  $\mu$ g of the total weight of the conjugated compound administered. In some preferred embodiments, conjugated compositions comprising *Pseudomonas* exotoxin are administered in doses in which the proportion by weight of *Pseudomonas* exotoxin is .1 - 10  $\mu$ g of the total weight of the conjugated compound administered. In some preferred embodiments, conjugated compositions comprising *Pseudomonas* exotoxin are administered in doses in which the proportion by weight of *Pseudomonas* exotoxin is .3 - 2.2  $\mu$ g of the total weight of the conjugated compound administered. The molecular weight of *Pseudomonas* exotoxin is 22,000. Thus, a conjugated compound that contains *Pseudomonas* exotoxin linked to an ST peptide has a molecular weight of about 23,300-24,500.

The range of doses of such conjugated compounds to be administered tested are .01 to 100  $\mu$ g. In some embodiments, .1 to 10  $\mu$ g of such conjugated compounds are administered. In some embodiments, .3 to 2.2  $\mu$ g of such conjugated compounds are administered.

To dose conjugated compositions comprising ST receptor binding moieties linked to active moieties that are radioisotopes in pharmaceutical compositions useful as imaging agents, it is presumed that each ST receptor binding moiety is linked to one radioactive active moiety. The amount of radioisotope to be administered is dependent upon the radioisotope. Those having ordinary skill in the art can readily formulate the amount of conjugated compound to be administered based upon the specific activity and energy of a given radionuclide used as an active moiety. Typically 0.1-100 millicuries per dose of imaging agent, preferably 1-10 millicuries, most often 2-5 millicuries are administered. Thus, pharmaceutical compositions according to the present invention useful as imaging agents which comprise conjugated compositions comprising an ST receptor binding moiety and a radioactive moiety comprise 0.1-100 millicuries, in some embodiments preferably 1-10 millicuries, in some embodiments preferably 2-5 millicuries, in some embodiments more preferably 1-5 millicuries. Examples of dosages include:  $^{131}\text{I}$  = between about 0.1-100 millicuries per dose, in some embodiments preferably 1-10 millicuries, in some embodiments 2-5 millicuries, and in some embodiments about 4 millicuries;  $^{111}\text{In}$  = between about 0.1-100 millicuries per dose, in some embodiments preferably 1-10 millicuries, in some embodiments 1-5 millicuries, and in some embodiments about 2 millicuries;  $^{99\text{m}}\text{Tc}$  = between about 0.1-100 millicuries per dose, in some embodiments preferably 5-75 millicuries, in some embodiments 10-50 millicuries, and in some embodiments about 27 millicuries. Depending upon the specific activity of the radioactive moiety and the weight of the ST receptor binding moiety the dosage defined by weight varies. ST peptides have molecular weights of between about 1300-2500. In the

pharmaceutical composition comprising an ST peptide linked to a single  $^{131}\text{I}$  in which the specific activity of  $^{131}\text{I}$ -ST peptide is about 2000 Ci/mmol, administering the dose of 0.1-100 millicuries is the equivalent of 0.1-100  $\mu\text{g}$   $^{131}\text{I}$ -ST peptide, 5 administering the dose of 1-10 millicuries is the equivalent of 1-10  $\mu\text{g}$  of  $^{131}\text{I}$ -ST peptide, administering the dose of 2-5 millicuries is equivalent to giving 2-5  $\mu\text{g}$  of  $^{131}\text{I}$ -ST peptide and administering the dose of 1-5 millicuries is equivalent to giving 1-5  $\mu\text{g}$  of  $^{131}\text{I}$ -ST peptide. In the pharmaceutical 10 composition comprising an ST peptide linked to a single  $^{111}\text{In}$  in which the specific activity of  $^{111}\text{In}$ -ST peptide is about 1 Ci/mmol, administering the dose of 0.1-100 millicuries is the equivalent of 0.2-200 mg  $^{111}\text{In}$ -ST peptide, administering the dose of 1-10 millicuries is the equivalent of 2-20 mg of  $^{111}\text{In}$ -ST 15 peptide, administering the dose of 2-5 millicuries is equivalent to giving 4-10 mg of  $^{111}\text{In}$ -ST peptide and administering the dose of 1-5 millicuries is equivalent to giving 2-10 mg of  $^{111}\text{In}$ -ST peptide.

To dose conjugated compositions comprising ST receptor 20 binding moieties linked to active moieties that are radioisotopes in pharmaceutical compositions useful as therapeutic agents, it is presumed that each ST receptor binding moiety is linked to one radioactive active moiety. The amount of radioisotope to be administered is dependent upon the 25 radioisotope. Those having ordinary skill in the art can readily formulate the amount of conjugated compound to be administered based upon the specific activity and energy of a given radionuclide used as an active moiety. For therapeutics that comprise  $^{131}\text{I}$ , between 10-1000 nM, preferably 50-500, more 30 preferably about 300 nanomoles of  $^{131}\text{I}$  at the tumor, per gram of tumor, is desirable. Thus, if there is about 1 gram of tumor, and about 0.1% of the administered dose binds to the tumor, 0.5-100 mg of  $^{131}\text{I}$ -ST peptide conjugated compound is administered. In some embodiments, 1 to 50 mg of  $^{131}\text{I}$ -ST 35 peptide conjugated compound is administered. In some embodiments, 5 to 10 mg of  $^{131}\text{I}$ -ST peptide conjugated compound is administered. Wessels B.W. and R.D. Rogus (1984) *Med. Phys.*

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11:638 and Kwok, C.S. et al. (1985) *Med. Phys.* 12:405, both of which are incorporated herein by reference, disclose detailed dose calculations for diagnostic and therapeutic conjugates which may be used in the preparation of pharmaceutical compositions of the present invention which include radioactive conjugated compounds.

One aspect of the present invention relates to a method of treating individuals suspected of suffering from metastasized colorectal cancer. Such individuals may be treated by administering to the individual a pharmaceutical composition that comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor binding moiety and an active moiety wherein the active moiety is a radiostable therapeutic agent. In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor binding moiety and an active moiety wherein the active moiety is a radiostable active agent and the ST receptor binding moiety is a peptide. In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor binding moiety and an active moiety wherein the active moiety is a radiostable active agent and the ST receptor binding moiety is selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NOS:5-54 and fragments and derivatives thereof. In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor binding moiety and an active moiety wherein the active moiety is a radiostable active agent and the ST receptor binding moiety is selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:54. In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor



binding moiety and an active moiety wherein the active moiety is a radiostable active agent selected from the group consisting of: methotrexate, doxorubicin, daunorubicin, cytosinarabioside, *cis*-platin, vindesine, mitomycin and  
5 bleomycin, alkaline phosphatase, ricin A chain, *Pseudomonas* exotoxin and diphtheria toxin. In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor binding moiety and an  
10 active moiety wherein the ST receptor binding moiety is selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:54 and the active moiety is a radiostable active agent selected from the group consisting of: methotrexate, doxorubicin, daunorubicin,  
15 cytosinarabioside, *cis*-platin, vindesine, mitomycin and bleomycin, alkaline phosphatase, ricin A chain, *Pseudomonas* exotoxin and diphtheria toxin. In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a  
20 radiostable conjugated compound described in Example 1. The individual being treated may be diagnosed as having metastasized colorectal cancer or may be diagnosed as having localized colorectal cancer and may undergo the treatment proactively in the event that there is some metastasis as yet  
25 undetected. The pharmaceutical composition contains a therapeutically effective amount of the conjugated composition. A therapeutically effective amount is an amount which is effective to cause a cytotoxic or cytostatic effect on metastasized colorectal cancer cells without causing lethal  
30 side effects on the individual.

One aspect of the present invention relates to a method of treating individuals suspected of suffering from metastasized colorectal cancer. Such individuals may be treated by administering to the individual a pharmaceutical  
35 composition that comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor binding moiety and an active moiety wherein the

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active moiety is a radioactive. In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor binding moiety and an active moiety wherein the active moiety is a radioactive and the ST receptor binding moiety is a peptide. In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor binding moiety and an active moiety wherein the active moiety is a radioactive and the ST receptor binding moiety is selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NOS:5-54 and fragments and derivatives thereof. In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor binding moiety and an active moiety wherein the active moiety is a radioactive and the ST receptor binding moiety is selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:54. In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor binding moiety and an active moiety wherein the active moiety is a radioactive agent selected from the group consisting of: <sup>47</sup>Sc, <sup>67</sup>Cu, <sup>90</sup>Y, <sup>109</sup>Pd, <sup>123</sup>I, <sup>125</sup>I, <sup>131</sup>I, <sup>186</sup>Re, <sup>188</sup>Re, <sup>199</sup>Au, <sup>211</sup>At, <sup>212</sup>Pb, <sup>212</sup>B, <sup>32</sup>P and <sup>33</sup>P, <sup>71</sup>Ge, <sup>77</sup>As, <sup>103</sup>Pb, <sup>105</sup>Rh, <sup>111</sup>Ag, <sup>119</sup>Sb, <sup>121</sup>Sn, <sup>131</sup>Cs, <sup>143</sup>Pr, <sup>161</sup>Tb, <sup>177</sup>Lu, <sup>191</sup>Os, <sup>193</sup>Mt and <sup>197</sup>Hg. In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor binding moiety and an active moiety wherein the ST receptor binding moiety is selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NOS:5-54 and fragments and derivatives thereof and the active moiety is a radioactive agent selected from the group consisting of: <sup>47</sup>Sc, <sup>67</sup>Cu, <sup>90</sup>Y, <sup>109</sup>Pd, <sup>123</sup>I, <sup>125</sup>I, <sup>131</sup>I, <sup>186</sup>Re, <sup>188</sup>Re, <sup>199</sup>Au, <sup>211</sup>At, <sup>212</sup>Pb, <sup>212</sup>B, <sup>32</sup>P and <sup>33</sup>P, <sup>71</sup>Ge, <sup>77</sup>As, <sup>103</sup>Pb, <sup>105</sup>Rh, <sup>111</sup>Ag, <sup>119</sup>Sb, <sup>121</sup>Sn, <sup>131</sup>Cs, <sup>143</sup>Pr



<sup>161</sup>Tb, <sup>177</sup>Lu, <sup>191</sup>Os, <sup>193M</sup>Pt, <sup>197</sup>Hg, <sup>32</sup>P and <sup>33</sup>P, <sup>71</sup>Ge, <sup>77</sup>As, <sup>103</sup>Pb, <sup>105</sup>Rh, <sup>111</sup>Ag, <sup>119</sup>Sb, <sup>121</sup>Sn, <sup>131</sup>Cs, <sup>143</sup>Pr, <sup>161</sup>Tb, <sup>177</sup>Lu, <sup>191</sup>Os, <sup>193M</sup>Pt, <sup>197</sup>Hg, all beta negative and/or auger emitters. In some embodiments of the present invention, the pharmaceutical composition comprises

5 a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor binding moiety and an active moiety wherein the active moiety is a radioactive agent selected from the group consisting of: <sup>47</sup>Sc, <sup>67</sup>Cu, <sup>90</sup>Y, <sup>109</sup>Pd, <sup>123</sup>I, <sup>125</sup>I, <sup>131</sup>I, <sup>186</sup>Re, <sup>188</sup>Re, <sup>199</sup>Au, <sup>211</sup>At, <sup>212</sup>Pb, <sup>212</sup>B, <sup>32</sup>P and <sup>33</sup>P, <sup>71</sup>Ge, <sup>77</sup>As, <sup>103</sup>Pb, <sup>105</sup>Rh, <sup>111</sup>Ag, <sup>119</sup>Sb, <sup>121</sup>Sn, <sup>131</sup>Cs, <sup>143</sup>Pr, <sup>161</sup>Tb, <sup>177</sup>Lu, <sup>191</sup>Os, <sup>193M</sup>Pt and <sup>197</sup>Hg. In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a conjugated

15 active moiety wherein the ST receptor binding moiety is selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:54 and the active moiety is a radioactive agent selected from the group consisting of: <sup>47</sup>Sc, <sup>67</sup>Cu, <sup>90</sup>Y, <sup>109</sup>Pd, <sup>123</sup>I, <sup>125</sup>I, <sup>131</sup>I, <sup>186</sup>Re, <sup>188</sup>Re, <sup>199</sup>Au, <sup>211</sup>At, <sup>212</sup>Pb, <sup>212</sup>B, <sup>32</sup>P and <sup>33</sup>P, <sup>71</sup>Ge, <sup>77</sup>As, <sup>103</sup>Pb, <sup>105</sup>Rh, <sup>111</sup>Ag, <sup>119</sup>Sb, <sup>121</sup>Sn, <sup>131</sup>Cs, <sup>143</sup>Pr, <sup>161</sup>Tb, <sup>177</sup>Lu, <sup>191</sup>Os, <sup>193M</sup>Pt and <sup>197</sup>Hg. In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a radioactive conjugated compound described in

20 Example 1. The individual being treated may be diagnosed as having metastasized colorectal cancer or may be diagnosed as having localized colorectal cancer and may undergo the treatment proactively in the event that there is some metastasis as yet undetected. The pharmaceutical composition

25 contains a therapeutically effective amount of the conjugated composition. A therapeutically effective amount is an amount which is effective to cause a cytotoxic or cytostatic effect on metastasized colorectal cancer cells without causing lethal side effects on the individual.

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35 One aspect of the present invention relates to a method of detecting metastasized colorectal cancer cells in an individual suspected of suffering from metastasized colorectal

cancer by radioimaging. Such individuals may be diagnosed as suffering from metastasized colorectal cancer and the metastasized colorectal cancer cells may be detected by administering to the individual, preferably by intravenous administration, a pharmaceutical composition that comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor binding moiety and an active moiety wherein the active moiety is a radioactive and detecting the presence of a localized accumulation or aggregation of radioactivity, indicating the presence of cells with ST receptors. In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor binding moiety and an active moiety wherein the active moiety is a radioactive and the ST receptor binding moiety is a peptide. In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor binding moiety and an active moiety wherein the active moiety is a radioactive and the ST receptor binding moiety is selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NOS:5-54 and fragments and derivatives thereof. In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor binding moiety and an active moiety wherein the active moiety is a radioactive and the ST receptor binding moiety is selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:54. In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor binding moiety and an active moiety wherein the active moiety is a radioactive agent selected from the group consisting of: radioactive heavy metals such as iron chelates, radioactive chelates of gadolinium or manganese, positron emitters of oxygen, nitrogen, iron, carbon,

or gallium,  $^{43}\text{K}$ ,  $^{52}\text{Fe}$ ,  $^{57}\text{Co}$ ,  $^{67}\text{Cu}$ ,  $^{67}\text{Ga}$ ,  $^{68}\text{Ga}$ ,  $^{77}\text{Br}$ ,  $^{81}\text{Rb}/^{81\text{M}}\text{Kr}$ ,  $^{87\text{M}}\text{Sr}$ ,  $^{99\text{M}}\text{Tc}$ ,  $^{111}\text{In}$ ,  $^{113\text{M}}\text{In}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{127}\text{Cs}$ ,  $^{129}\text{Cs}$ ,  $^{131}\text{I}$ ,  $^{132}\text{I}$ ,  $^{197}\text{Hg}$ ,  $^{203}\text{Pb}$  and  $^{206}\text{Bi}$ . In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor binding moiety and an active moiety wherein the ST receptor binding moiety is selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NOS:5-54 and fragments and derivatives thereof and the active moiety is a radioactive agent selected from the group consisting of: radioactive heavy metals such as iron chelates, radioactive chelates of gadolinium or manganese, positron emitters of oxygen, nitrogen, iron, carbon, or gallium,  $^{43}\text{K}$ ,  $^{52}\text{Fe}$ ,  $^{57}\text{Co}$ ,  $^{67}\text{Cu}$ ,  $^{67}\text{Ga}$ ,  $^{68}\text{Ga}$ ,  $^{77}\text{Br}$ ,  $^{81}\text{Rb}/^{81\text{M}}\text{Kr}$ ,  $^{87\text{M}}\text{Sr}$ ,  $^{99\text{M}}\text{Tc}$ ,  $^{111}\text{In}$ ,  $^{113\text{M}}\text{In}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{127}\text{Cs}$ ,  $^{129}\text{Cs}$ ,  $^{131}\text{I}$ ,  $^{132}\text{I}$ ,  $^{197}\text{Hg}$ ,  $^{203}\text{Pb}$  and  $^{206}\text{Bi}$ . In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor binding moiety and an active moiety wherein the active moiety is a radioactive agent selected from the group consisting of:  $^{43}\text{K}$ ,  $^{52}\text{Fe}$ ,  $^{57}\text{Co}$ ,  $^{67}\text{Cu}$ ,  $^{67}\text{Ga}$ ,  $^{68}\text{Ga}$ ,  $^{77}\text{Br}$ ,  $^{81}\text{Rb}/^{81\text{M}}\text{Kr}$ ,  $^{87\text{M}}\text{Sr}$ ,  $^{99\text{M}}\text{Tc}$ ,  $^{111}\text{In}$ ,  $^{113\text{M}}\text{In}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{127}\text{Cs}$ ,  $^{129}\text{Cs}$ ,  $^{131}\text{I}$ ,  $^{132}\text{I}$ ,  $^{197}\text{Hg}$ ,  $^{203}\text{Pb}$  and  $^{206}\text{Bi}$ . In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a conjugated compound that comprises an ST receptor binding moiety and an active moiety wherein the ST receptor binding moiety is selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:54 and the active moiety is a radioactive agent selected from the group consisting of:  $^{43}\text{K}$ ,  $^{52}\text{Fe}$ ,  $^{57}\text{Co}$ ,  $^{67}\text{Cu}$ ,  $^{67}\text{Ga}$ ,  $^{68}\text{Ga}$ ,  $^{77}\text{Br}$ ,  $^{81}\text{Rb}/^{81\text{M}}\text{Kr}$ ,  $^{87\text{M}}\text{Sr}$ ,  $^{99\text{M}}\text{Tc}$ ,  $^{111}\text{In}$ ,  $^{113\text{M}}\text{In}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{127}\text{Cs}$ ,  $^{129}\text{Cs}$ ,  $^{131}\text{I}$ ,  $^{132}\text{I}$ ,  $^{197}\text{Hg}$ ,  $^{203}\text{Pb}$  and  $^{206}\text{Bi}$ . In some embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier or diluent and a radioactive conjugated compound described in Example 1. The individual being treated may be diagnosed as having metastasized colorectal cancer or may be diagnosed as having

localized colorectal cancer and may undergo the treatment proactively in the event that there is some metastasis as yet undetected. The pharmaceutical composition contains a diagnostically effective amount of the conjugated composition.

5 A diagnostically effective amount is an amount which can be detected at a site in the body where cells with ST receptors are located without causing lethal side effects on the individual.

Another aspect of the invention relates to unconjugated compositions which comprise an ST receptor binding ligand and an active agent. For example, liposomes are small vesicles composed of lipids. Drugs can be introduced into the center of these vesicles. The outer shell of these vesicles comprise an ST receptor binding ligand. *Liposomes* Volumes 1, 2 and 3 CRC Press Inc. Boca Raton FLA, which is incorporated herein by reference, disclose preparation of liposome-encapsulated active agents which include targeting agents that correspond to ST receptor ligand in the outer shell. Unconjugated compositions which comprise an ST receptor ligand in the matrix of a liposome with an active agent inside include such compositions in which the ST receptor ligand is selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NOS:5-54 and fragments and derivatives thereof and the active agent is selected from the group consisting of: methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-fluorouracil, melphalan, chlorambucil, *cis*-platinum, vindesine, mitomycin, bleomycin, purothionin, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, *Pseudomonas* exotoxin, diphtheria toxin, *Clostridium perfringens* phospholipase C, bovine pancreatic ribonuclease, pokeweed antiviral protein, abrin, abrin A chain, cobra venom factor, gelonin, saporin, modeccin, viscumin, volkensin, alkaline phosphatase, nitroimidazole, metronidazole and misonidazole.

Another aspect of the invention relates to  
35 unconjugated and conjugated compositions which comprise an ST  
receptor ligand used to deliver therapeutic nucleic acid  
molecules to cells that comprise an ST receptor such as normal

cells of the intestinal tract as well as metastasized colorectal cancer cells. In some embodiments, the genetic material is delivered to metastasized tumor cells to produce an antigen that can be targeted by the immune system or to produce a protein which kills the cell or inhibits its proliferation. In some embodiments, the ST receptor ligand is used to deliver nucleic acids that encode nucleic acid molecules which replace defective endogenous genes or which encode therapeutic proteins. In some embodiments, the ST receptor ligand is thus used to deliver the active agent specifically to the cells lining the intestinal tract to treat diseases specific to this organ. According to this aspect of the invention, compositions comprise nucleic acid molecules which can replace defective genes. In some embodiments, the compositions are used in gene therapy protocols to deliver to individuals, genetic material needed and/or desired to make up for a genetic deficiency.

In some embodiments, the ST receptor ligand is combined with or incorporated into a delivery vehicle thereby converting the delivery vehicle into a specifically targeted delivery vehicle. For example, an ST receptor binding peptide may be integrated into the outer portion of a viral particle making such a virus an ST receptor-bearing cell specific virus. Similarly, the coat protein of a virus may be engineered such that it is produced as a fusion protein which includes an active ST receptor binding peptide that is exposed or otherwise accessible on the outside of the viral particle making such a virus an ST receptor-bearing cell-specific virus. In some embodiments, an ST receptor ligand may be integrated or otherwise incorporated into the liposomes wherein the ST receptor ligand is exposed or otherwise accessible on the outside of the liposome making such liposomes specifically targeted to ST receptor-bearing cells.

The active agent in the conjugated or unconjugated compositions according to this aspect of the invention is a  
35 nucleic acid molecule. The nucleic acid may be RNA or preferably DNA. In some embodiments, the nucleic acid molecule is an antisense molecule or encodes an antisense sequence whose

presence in the cell inhibits production of an undesirable protein. In some embodiments, the nucleic acid molecule encodes a ribozyme whose presence in the cell inhibits production of an undesirable protein. In some embodiments, the  
5 nucleic acid molecule encodes a protein or peptide that is desirably produced in the cell. In some embodiments, the nucleic acid molecule encodes a functional copy of a gene that is defective in the targeted cell. The nucleic acid molecule is preferably operably linked to regulatory elements needed to  
10 express the coding sequence in the cell.

Liposomes are small vesicles composed of lipids. Genetic constructs which encode proteins that are desired to be expressed in ST receptor-bearing cells are introduced into the center of these vesicles. The outer shell of these vesicles  
15 comprise an ST receptor ligand, in some embodiments preferably an ST peptide. *Liposomes* Volumes 1, 2 and 3 CRC Press Inc. Boca Raton FLA, which is incorporated herein by reference, disclose preparation of liposome-encapsulated active agents which include antibodies in the outer shell. In the present  
20 invention, an ST receptor ligand such as for example an ST peptide corresponds to the antibodies in the outer shell. Unconjugated compositions which comprise an ST receptor ligand in the matrix of a liposome with an active agent inside include such compositions in which the ST receptor ligand is selected  
25 from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NOS:5-54 and fragments and derivatives thereof.

In one embodiment for example, cystic fibrosis, a genetic disease in which there is a mutation of a specific gene encoding a chloride transport protein which ultimately produces  
30 abnormalities of function in many systems, most notably in the respiratory and intestinal tract, is treated by gene therapy techniques using ST receptor ligands to deliver the corrective gene to cells. Current therapy has been directed at replacing the mutant gene in the respiratory system with the normal gene  
35 by targeting these genes directly to the cells lining the respiratory tract using viruses which bind only to those cells. Similarly, the normal gene is packaged in liposomes targeted on

their surface with ST receptor ligands and delivered to the intestinal tract. ST receptor ligands specifically target and direct the liposomes containing the normal gene to correct the lesion for cystic fibrosis to the specific cells lining the  
5 intestinal tract, from the duodenum to the rectum. Uptake of that genetic material by those cells should result in a cure of cystic fibrosis in the intestinal tract.

In another embodiment, the delivery of normal copies of the p53 tumor suppressor gene to the intestinal tract is  
10 accomplished using ST receptor ligand to target the gene therapeutic. Mutations of the p53 tumor suppressor gene appears to play a prominent role in the development of colorectal cancer in the intestinal tract. One approach to combatting this disease is the delivery of normal copies of  
15 this gene to the intestinal tract to cells expressing mutant forms of this gene. Genetic constructs that comprise normal p53 tumor suppressor genes are incorporated into liposomes that comprise an ST receptor ligand. The composition is delivered to the intestinal tract. ST receptor binding ligands  
20 specifically target and direct the liposomes containing the normal gene to correct the lesion created by mutation of p53 suppressor gene in intestinal cells.

Preparation of genetic constructs is with the skill of those having ordinary skill in the art. The present  
25 invention allows such construct to be specifically targeted by using the ST receptor ligands of the present invention. The compositions of the invention include an ST receptor ligand such as an ST peptide associated with a delivery vehicle and a gene construct which comprises a coding sequence for a protein  
30 whose production is desired in the cells of the intestinal tract linked to necessary regulatory sequences for expression in the cells. For uptake by cells of the intestinal tract, the compositions are administered orally or by enema whereby they enter the intestinal tract and contact cells which comprise ST  
35 receptors. The delivery vehicles associate with the ST receptor by virtue of the ST receptor ligand and the vehicle is internalized into the cell or the active agent/genetic

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construct is otherwise taken up by the cell. Once internalized, the construct can provide a therapeutic effect on the individual. One having ordinary skill in the art can readily formulate such compositions for oral or enema  
5 administration and determine the effective amount of such composition to be administered to treat the disease or disorder.

The following examples are illustrative but are not meant to be limiting of the present invention.

## 10 Examples

### Example 1

The following are representative compounds according to the present invention. Whenever stated below, reference to a series of compounds is provided for efficiency and is meant  
15 to name each compound in the series including all the compounds in numerical order, such as for example "3-D1 to 3-D16" is meant to refer to compounds 3-D1, 3-D2, 3-D3, 3-D4, 3-D5, 3-D6, 3-D7, 3-D8, 3-D9, 3-D10, 3-D11, 3-D12, 3-D13, 3-D14, 3-D15 and 3-D16. Likewise, whenever stated below, reference to a series  
20 of SEQ ID NO:'s is provided for efficiency and is meant to name each SEQ ID NO: in the series including the all SEQ ID NO:'s in numerical order, such as for example SEQ ID NO:5 through SEQ ID NO:54 is meant to refer to SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ  
25 ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID  
30 NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53 and SEQ ID NO:54. Similarly, whenever  
35 stated below, reference to a series of compounds is provided for efficiency and is meant to name each compound in the series



including the all compounds in numerical order, such as for example "5-AP to 54-AP" is meant to refer to compounds 5-AP, 6-AP, 7-AP, 8-AP, 9-AP, 10-AP, 11-AP, 12-AP, 13-AP, 14-AP, 15-AP, 16-AP, 17-AP, 18-AP, 19-AP, 20-AP, 21-AP, 22-AP, 23-AP, 24-AP, 25-AP, 26-AP, 27-AP, 28-AP, 29-AP, 30-AP, 31-AP, 32-AP, 33-AP, 34-AP, 35-AP, 36-AP, 37-AP, 38-AP, 39-AP, 40-AP, 41-AP, 42-AP, 43-AP, 44-AP, 45-AP, 46-AP, 47-AP, 48-AP, 49-AP, 50-AP, 51-AP, 52-AP, 53-AP and 54-AP.

Compound 2-D1 comprises methotrexate (amethopterin)  
10 conjugated to SEQ ID NO:2.

Compound 2-D2 comprises doxorubicin (adrimycin) conjugated to SEQ ID NO:2.

Compound 2-D3 comprises daunorubicin conjugated to SEQ  
ID NO:2.

15           Compound 2-D4 comprises cytosinarabinoside conjugated  
to SEO ID NO:2.

Compound 2-D5 comprises etoposide conjugated to SEQ  
ID NO:2.

Compound 2-D6 comprises 5-4 fluorouracil conjugated  
20 to SEQ ID NO:2.

Compound 2-D7 comprises melphalan conjugated to SEQ  
ID NO:2.

Compound 2-D8 comprises chlorambucil conjugated to SEQ  
ID NO:2.

25           Compound 2-D9 comprises cyclophosphamide conjugated  
to SEQ ID NO:2.

Compound 2-D10 comprises *cis*-platinum conjugated to  
 SEO ID NO:2.

Compound 2-D11 comprises vindesine conjugated to SEQ  
30 ID NO:2.

Compound 2-D12 comprises mitomycin conjugated to SEQ  
ID NO:2.

Compound 2-D13 comprises bleomycin conjugated to SEQ  
ID NO:2.

35                   Compound 2-D14 comprises purothionin conjugated to SEQ  
ID NO:2.

[illegible]

Compound 2-D15 comprises macromomycin conjugated to  
SEQ ID NO:2.

Compound 2-D16 comprises trenimon conjugated to SEQ  
ID NO:2.

5           Compounds 3-D1 to 3-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 3-D1 to 3-D16 each comprise SEQ ID NO:3 as the ST receptor binding moiety.

Compounds 5-D1 to 5-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 5-D1 to 5-D16 each comprise SEQ ID NO:5 as the ST receptor binding moiety.

Compounds 6-D1 to 6-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 6-D1 to 6-D16 each comprise SEQ ID NO:6 as the ST receptor binding moiety.

Compounds 7-D1 to 7-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 7-D1 to 7-D16 each comprise SEQ ID NO:7 as the ST receptor binding moiety.

Compounds 8-D1 to 8-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 8-D1 to 8-D16 each comprise SEQ ID NO:8 as the ST receptor binding moiety.

25           Compounds 9-D1 to 9-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 9-D1 to 9-D16 each comprise SEQ ID NO:9 as the ST receptor binding moiety.

Compounds 10-D1 to 10-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 10-D1 to 10-D16 each comprise SEQ ID NO:2 as the ST receptor binding moiety.

Compounds 12-D1 to 12-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 12-D1 to

12-D16 each comprise SEQ ID NO:11 as the ST receptor binding moiety.

Compounds 12-D1 to 12-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 12-D1 to 12-D16 each comprise SEQ ID NO:12 as the ST receptor binding moiety.

Compounds 13-D1 to 13-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 13-D1 to 13-D16 each comprise SEQ ID NO:13 as the ST receptor binding moiety.

Compounds 14-D1 to 14-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 14-D1 to 14-D16 each comprise SEQ ID NO:14 as the ST receptor binding moiety.

Compounds 15-D1 to 15-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 15-D1 to 15-D16 each comprise SEQ ID NO:15 as the ST receptor binding moiety.

Compounds 16-D1 to 16-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 16-D1 to 16-D16 each comprise SEQ ID NO:16 as the ST receptor binding moiety.

Compounds 17-D1 to 17-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 17-D1 to 17-D16 each comprise SEQ ID NO:17 as the ST receptor binding moiety.

Compounds 18-D1 to 18-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 18-D1 to 18-D16 each comprise SEQ ID NO:18 as the ST receptor binding moiety.

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Compounds 19-D1 to 19-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 19-D1 to 19-D16 each comprise SEQ ID NO:19 as the ST receptor binding  
5 moiety.

Compounds 20-D1 to 20-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 20-D1 to 20-D16 each comprise SEQ ID NO:20 as the ST receptor binding  
10 moiety.

Compounds 22-D1 to 22-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 22-D1 to 22-D16 each comprise SEQ ID NO:21 as the ST receptor binding  
15 moiety.

Compounds 22-D1 to 22-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 22-D1 to 22-D16 each comprise SEQ ID NO:22 as the ST receptor binding  
20 moiety.

Compounds 23-D1 to 23-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 23-D1 to 23-D16 each comprise SEQ ID NO:23 as the ST receptor binding  
25 moiety.

Compounds 24-D1 to 24-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 24-D1 to 24-D16 each comprise SEQ ID NO:24 as the ST receptor binding  
30 moiety.

Compounds 25-D1 to 25-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 25-D1 to 25-D16 each comprise SEQ ID NO:25 as the ST receptor binding  
35 moiety.

Compounds 26-D1 to 26-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ

ID NO:2 as the ST receptor binding moiety, compounds 26-D1 to 26-D16 each comprise SEQ ID NO:26 as the ST receptor binding moiety.

Compounds 27-D1 to 27-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 27-D1 to 27-D16 each comprise SEQ ID NO:27 as the ST receptor binding moiety.

Compounds 28-D1 to 28-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 28-D1 to 28-D16 each comprise SEQ ID NO:28 as the ST receptor binding moiety.

Compounds 29-D1 to 29-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 29-D1 to 29-D16 each comprise SEQ ID NO:29 as the ST receptor binding moiety.

Compounds 30-D1 to 30-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 30-D1 to 30-D16 each comprise SEQ ID NO:30 as the ST receptor binding moiety.

Compounds 32-D1 to 32-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 32-D1 to 32-D16 each comprise SEQ ID NO:31 as the ST receptor binding moiety.

Compounds 32-D1 to 32-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 32-D1 to 32-D16 each comprise SEQ ID NO:32 as the ST receptor binding moiety.

Compounds 33-D1 to 33-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 33-D1 to

33-D16 each comprise SEQ ID NO:33 as the ST receptor binding moiety.

Compounds 34-D1 to 34-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 34-D1 to 34-D16 each comprise SEQ ID NO:34 as the ST receptor binding moiety.

Compounds 35-D1 to 35-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 35-D1 to 35-D16 each comprise SEQ ID NO:35 as the ST receptor binding moiety.

Compounds 36-D1 to 36-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 36-D1 to 36-D16 each comprise SEQ ID NO:36 as the ST receptor binding moiety.

Compounds 37-D1 to 37-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 37-D1 to 37-D16 each comprise SEQ ID NO:37 as the ST receptor binding moiety.

Compounds 38-D1 to 38-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 38-D1 to 38-D16 each comprise SEQ ID NO:38 as the ST receptor binding moiety.

Compounds 39-D1 to 39-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 39-D1 to 39-D16 each comprise SEQ ID NO:39 as the ST receptor binding moiety.

Compounds 40-D1 to 40-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 40-D1 to 40-D16 each comprise SEQ ID NO:40 as the ST receptor binding moiety.

Compounds 42-D1 to 42-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 42-D1 to 42-D16 each comprise SEQ ID NO:41 as the ST receptor binding  
5 moiety.

Compounds 42-D1 to 42-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 42-D1 to 42-D16 each comprise SEQ ID NO:42 as the ST receptor binding  
10 moiety.

Compounds 43-D1 to 43-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 43-D1 to 43-D16 each comprise SEQ ID NO:43 as the ST receptor binding  
15 moiety.

Compounds 44-D1 to 44-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 44-D1 to 44-D16 each comprise SEQ ID NO:44 as the ST receptor binding  
20 moiety.

Compounds 45-D1 to 45-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 45-D1 to 45-D16 each comprise SEQ ID NO:45 as the ST receptor binding  
25 moiety.

Compounds 46-D1 to 46-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 46-D1 to 46-D16 each comprise SEQ ID NO:46 as the ST receptor binding  
30 moiety.

Compounds 47-D1 to 47-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 47-D1 to 47-D16 each comprise SEQ ID NO:47 as the ST receptor binding  
35 moiety.

Compounds 48-D1 to 48-D16 are the same as compounds 2-D1 to 2-D16, respectively, except instead of comprising SEQ

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ID NO:2 as the ST receptor binding moiety, compounds 48-D1 to 48-D16 each comprise SEQ ID NO:48 as the ST receptor binding moiety.

Compounds 49-D1 to 49-D16 are the same as compounds 5 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 49-D1 to 49-D16 each comprise SEQ ID NO:49 as the ST receptor binding moiety.

Compounds 50-D1 to 50-D16 are the same as compounds 10 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 50-D1 to 50-D16 each comprise SEQ ID NO:50 as the ST receptor binding moiety.

Compounds 51-D1 to 51-D16 are the same as compounds 15 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 51-D1 to 51-D16 each comprise SEQ ID NO:51 as the ST receptor binding moiety.

Compounds 52-D1 to 52-D16 are the same as compounds 20 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 52-D1 to 52-D16 each comprise SEQ ID NO:52 as the ST receptor binding moiety.

Compounds 53-D1 to 53-D16 are the same as compounds 25 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 53-D1 to 53-D16 each comprise SEQ ID NO:53 as the ST receptor binding moiety.

Compounds 54-D1 to 54-D16 are the same as compounds 30 2-D1 to 2-D16, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 54-D1 to 54-D16 each comprise SEQ ID NO:54 as the ST receptor binding moiety.

Compound 2-T1 comprises ricin conjugated to SEQ ID 35 NO:2.

Compound 2-T2 comprises ricin A chain (ricin toxin) conjugated to SEQ ID NO:2.

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**Figure 1: Effect of the 1997-1998 El Niño on the growth of 10-year-old trees in a tropical forest.**

**Map of the study area:** The map shows the location of the study area in the Amazon basin, with a scale bar indicating 100 km. The study area is located in the central Amazon, near the border of Brazil and Peru.

**Precipitation (mm) by month:** The bar chart shows monthly precipitation in mm for the years 1997, 1998, and 1999. The x-axis represents months from January to December. The y-axis represents precipitation in mm, ranging from 0 to 100. The bars are color-coded: blue for 1997, red for 1998, and green for 1999.

**Growth rate (mm year<sup>-1</sup>) by month:** The line graph shows the growth rate in mm year<sup>-1</sup> for the years 1997, 1998, and 1999. The x-axis represents months from January to December. The y-axis represents growth rate in mm year<sup>-1</sup>, ranging from 0 to 10. The lines are color-coded: blue for 1997, red for 1998, and green for 1999.

**Growth rate (mm year<sup>-1</sup>) by species:** The table shows the growth rate in mm year<sup>-1</sup> for different species in the years 1997, 1998, and 1999. The species are listed on the x-axis, and the growth rates are listed on the y-axis.

Species	1997	1998	1999
1	1.2	1.5	1.8
2	1.5	1.8	2.1
3	1.8	2.1	2.4
4	2.1	2.4	2.7
5	2.4	2.7	3.0
6	2.7	3.0	3.3
7	3.0	3.3	3.6
8	3.3	3.6	3.9
9	3.6	3.9	4.2
10	3.9	4.2	4.5

**Figure 1: Effect of the 1997-1998 El Niño on the growth of 10-year-old trees in a tropical forest.**

**Map of the study area:** The map shows the location of the study area in the Amazon basin, with a scale bar indicating 100 km. The study area is located in the central Amazon, near the border of Brazil and Peru.

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**Growth rate (mm year<sup>-1</sup>) by month:** The line graph shows the growth rate in mm year<sup>-1</sup> for the years 1997, 1998, and 1999. The x-axis represents months from January to December. The y-axis represents growth rate in mm year<sup>-1</sup>, ranging from 0 to 10. The lines are color-coded: blue for 1997, red for 1998, and green for 1999.

**Growth rate (mm year<sup>-1</sup>) by species:** The table shows the growth rate in mm year<sup>-1</sup> for different species in the years 1997, 1998, and 1999. The species are listed on the x-axis, and the growth rates are listed on the y-axis.

Species	1997	1998	1999
1	1.2	1.5	1.8
2	1.5	1.8	2.1
3	1.8	2.1	2.4
4	2.1	2.4	2.7
5	2.4	2.7	3.0
6	2.7	3.0	3.3
7	3.0	3.3	3.6
8	3.3	3.6	3.9
9	3.6	3.9	4.2
10	3.9	4.2	4.5

**Figure 1: Effect of the 1997-1998 El Niño on the growth of 10-year-old trees in a tropical forest.**

**Map of the study area:** The map shows the location of the study area in the Amazon basin, with a scale bar indicating 100 km. The study area is located in the central Amazon, near the border of Brazil and Peru.

**Precipitation (mm) by month:** The bar chart shows monthly precipitation in mm for the years 1997, 1998, and 1999. The x-axis represents months from January to December. The y-axis represents precipitation in mm, ranging from 0 to 100. The bars are color-coded: blue for 1997, red for 1998, and green for 1999.

**Growth rate (mm year<sup>-1</sup>) by month:** The line graph shows the growth rate in mm year<sup>-1</sup> for the years 1997, 1998, and 1999. The x-axis represents months from January to December. The y-axis represents growth rate in mm year<sup>-1</sup>, ranging from 0 to 10. The lines are color-coded: blue for 1997, red for 1998, and green for 1999.

**Growth rate (mm year<sup>-1</sup>) by species:** The table shows the growth rate in mm year<sup>-1</sup> for different species in the years 1997, 1998, and 1999. The species are listed on the x-axis, and the growth rates are listed on the y-axis.

Species	1997	1998	1999
1	1.2	1.5	1.8
2	1.5	1.8	2.1
3	1.8	2.1	2.4
4	2.1	2.4	2.7
5	2.4	2.7	3.0
6	2.7	3.0	3.3
7	3.0	3.3	3.6
8	3.3	3.6	3.9
9	3.6	3.9	4.2
10	3.9	4.2	4.5

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14-T15 each comprise SEQ ID NO:14 as the ST receptor binding moiety.

Compounds 15-T1 to 15-T15 are the same as compounds 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 15-T1 to 15-T15 each comprise SEQ ID NO:15 as the ST receptor binding moiety.

Compounds 15-T1 to 15-T15 are the same as compounds 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 15-T1 to 15-T15 each comprise SEQ ID NO:15 as the ST receptor binding moiety.

Compounds 17-T1 to 17-T15 are the same as compounds 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 17-T1 to 17-T15 each comprise SEQ ID NO:17 as the ST receptor binding moiety.

Compounds 18-T1 to 18-T15 are the same as compounds 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 18-T1 to 18-T15 each comprise SEQ ID NO:18 as the ST receptor binding moiety.

Compounds 19-T1 to 19-T15 are the same as compounds 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 19-T1 to 19-T15 each comprise SEQ ID NO:19 as the ST receptor binding moiety.

Compounds 20-T1 to 20-T15 are the same as compounds 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 20-T1 to 20-T15 each comprise SEQ ID NO:20 as the ST receptor binding moiety.

Compounds 21-T1 to 21-T15 are the same as compounds 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 21-T1 to 21-T15 each comprise SEQ ID NO:21 as the ST receptor binding moiety.

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5 moiety.

10 moiety.

15 moiety.

20 moiety.

25 moiety

30 moiety

35 moiety

2-T1 t

ID NO:2 as the ST receptor binding moiety, compounds 29-T1 to 29-T15 each comprise SEQ ID NO:29 as the ST receptor binding moiety.

Compounds 30-T1 to 30-T15 are the same as compounds 5 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 30-T1 to 30-T15 each comprise SEQ ID NO:30 as the ST receptor binding moiety.

Compounds 31-T1 to 31-T15 are the same as compounds 10 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 31-T1 to 31-T15 each comprise SEQ ID NO:31 as the ST receptor binding moiety.

Compounds 32-T1 to 32-T15 are the same as compounds 15 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 32-T1 to 32-T15 each comprise SEQ ID NO:32 as the ST receptor binding moiety.

Compounds 33-T1 to 33-T15 are the same as compounds 20 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 33-T1 to 33-T15 each comprise SEQ ID NO:33 as the ST receptor binding moiety.

Compounds 34-T1 to 34-T15 are the same as compounds 25 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 34-T1 to 34-T15 each comprise SEQ ID NO:34 as the ST receptor binding moiety.

Compounds 35-T1 to 35-T15 are the same as compounds 30 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 35-T1 to 35-T15 each comprise SEQ ID NO:35 as the ST receptor binding moiety.

Compounds 36-T1 to 36-T15 are the same as compounds 35 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 36-T1 to

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36-T15 each comprise SEQ ID NO:36 as the ST receptor binding moiety.

Compounds 37-T1 to 37-T15 are the same as compounds 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 37-T1 to 37-T15 each comprise SEQ ID NO:37 as the ST receptor binding moiety.

Compounds 38-T1 to 38-T15 are the same as compounds 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 38-T1 to 38-T15 each comprise SEQ ID NO:38 as the ST receptor binding moiety.

Compounds 39-T1 to 39-T15 are the same as compounds 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 39-T1 to 39-T15 each comprise SEQ ID NO:39 as the ST receptor binding moiety.

Compounds 40-T1 to 40-T15 are the same as compounds 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 40-T1 to 40-T15 each comprise SEQ ID NO:40 as the ST receptor binding moiety.

Compounds 41-T1 to 41-T15 are the same as compounds 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 41-T1 to 41-T15 each comprise SEQ ID NO:41 as the ST receptor binding moiety.

Compounds 42-T1 to 42-T15 are the same as compounds 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 42-T1 to 42-T15 each comprise SEQ ID NO:42 as the ST receptor binding moiety.

Compounds 43-T1 to 43-T15 are the same as compounds 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 43-T1 to 43-T15 each comprise SEQ ID NO:43 as the ST receptor binding moiety.



Compounds 52-T1 to 52-T15 are the same as compounds 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 52-T1 to 52-T15 each comprise SEQ ID NO:52 as the ST receptor binding moiety.

Compounds 54-T1 to 54-T15 are the same as compounds 2-T1 to 2-T15, respectively, except instead of comprising SEQ ID NO:2 as the ST receptor binding moiety, compounds 54-T1 to 54-T15 each comprise SEQ ID NO:54 as the ST receptor binding moiety.

Compounds 2-NIZ, 3-NIZ and 5-NIZ to 54-NIZ refer to the 51 conjugated compounds that comprise nitroimidazole  
25 conjugated to SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-MIS, 3-MIS and 5-MIS to 54-MIS refer to the 51 conjugated compounds that comprise misonidazole conjugated to SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:5 through SEQ ID NO:54, respectively.

35           Compounds 2-47Sc, 3-47Sc and 5-47Sc to 54-47Sc refer  
to the 51 conjugated compounds that comprise <sup>47</sup>Sc conjugated to



SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-67Cu, 3-67Cu and 5-67Cu to 54-67Cu refer to the 51 conjugated compounds that comprise <sup>67</sup>Cu conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-90Y, 3-90Y and 5-90Y to 54-90Y refer to the 51 conjugated compounds that comprise <sup>90</sup>Y conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-109Pd, 3-109Pd and 5-109Pd to 54-109Pd refer to the 51 conjugated compounds that comprise <sup>109</sup>Pd conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

15                   Compounds 2-123I, 3-123I and 5-123I to 54-123I refer to the 51 conjugated compounds that comprise <sup>123</sup>I conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-125I, 3-125I and 5-125I to 54-125I refer to the 51 conjugated compounds that comprise <sup>125</sup>I conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-131I, 3-131I and 5-131I to 54-131I refer to the 51 conjugated compounds that comprise <sup>131</sup>I conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-132I, 3-132I and 5-132I to 54-132I refer to the 51 conjugated compounds that comprise <sup>132</sup>I conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-186Re, 3-186Re and 5-186Re to 54-186Re refer to the 51 conjugated compounds that comprise <sup>186</sup>Re, conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

35                   Compounds 2-188Re, 3-188Re and 5-188Re to 54-188Re  
refer to the 51 conjugated compounds that comprise <sup>188</sup>Re,

conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-199Au, 3-199Au and 5-199Au to 54-199Au refer to the 51 conjugated compounds that comprise <sup>199</sup>Au, conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-211At, 3-211At and 5-211At to 54-211At refer to the 51 conjugated compounds that comprise <sup>211</sup>At, conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-212Pb, 3-212Pb and 5-212Pb to 54-212Pb refer to the 51 conjugated compounds that comprise <sup>212</sup>Pb conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-212Bi, 3-212Bi and 5-212Bi to 54-212Bi refer to the 51 conjugated compounds that comprise <sup>212</sup>Bi conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-203Pb, 3-203Pb and 5-203Pb to 54-203Pb refer to the 51 conjugated compounds that comprise <sup>203</sup>Pb conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-206Bi, 3-206Bi and 5-206Bi to 54-206Bi refer to the 51 conjugated compounds that comprise <sup>206</sup>Bi conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-32P, 3-32P and 5-32P to 54-32P refer to the 51 conjugated compounds that comprise <sup>32</sup>P conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-33P, 3-33P and 5-33P to 54-33P refer to the 51 conjugated compounds that comprise <sup>33</sup>P conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-71Ge, 3-71Ge and 5-71Ge to 54-71Ge refer to the 51 conjugated compounds that comprise <sup>71</sup>Ge conjugated to

SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-77As, 3-77As and 5-77As to 54-77As refer to the 51 conjugated compounds that comprise <sup>77</sup>As conjugated to  
5 SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-103Pd, 3-103Pd and 5-103Pd to 54-103Pd refer to the 51 conjugated compounds that comprise <sup>103</sup>Pd conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through  
10 SEQ ID NO:54, respectively.

Compounds 2-105Rh, 3-105Rh and 5-105Rh to 54-105Rh refer to the 51 conjugated compounds that comprise <sup>105</sup>Rh conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-111Ag, 3-111Ag and 5-111Ag to 54-111Ag refer to the 51 conjugated compounds that comprise <sup>111</sup>Ag conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through  
15 SEQ ID NO:54, respectively.

Compounds 2-119Sb, 3-119Sb and 5-119Sb to 54-119Sb refer to the 51 conjugated compounds that comprise <sup>119</sup>Sb conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through  
20 SEQ ID NO:54, respectively.

Compounds 2-121Sn, 3-121-Sn and 5-121Sn to 54-121Sn refer to the 51 conjugated compounds that comprise <sup>121</sup>Sn conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through  
25 SEQ ID NO:54, respectively.

Compounds 2-131Cs, 3-131Cs and 5-131Cs to 54-131Cs refer to the 51 conjugated compounds that comprise <sup>131</sup>Cs conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through  
30 SEQ ID NO:54, respectively.

Compounds 2-127Cs, 3-131Cs and 5-131Cs to 54-127Cs refer to the 51 conjugated compounds that comprise <sup>127</sup>Cs conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through  
SEQ ID NO:54, respectively.

Compounds 2-129Cs, 3-129Cs and 5-129Cs to 54-129Cs refer to the 51 conjugated compounds that comprise <sup>129</sup>Cs  
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conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through  
SEQ ID NO:54, respectively.

Compounds 2-143Pr, 3-143Pr and 5-143Pr to 54-143Pr refer to the 51 conjugated compounds that comprise <sup>143</sup>Pr 5 conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-161Tb, 3-161Tb and 5-161Tb to 54-161Tb refer to the 51 conjugated compounds that comprise <sup>161</sup>Tb conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-177Lu, 3-177Lu and 5-177Lu to 54-177Lu refer to the 51 conjugated compounds that comprise <sup>177</sup>Lu conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

15           Compounds 2-1910s, 3-1910s and 5-1910s to 54-1910s  
refer to the 51 conjugated compounds that comprise <sup>191</sup>Os  
conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through  
SEQ ID NO:54, respectively.

Compounds 2-193mPt, 3-193mPt and 5-193mPt to 54-193mPt  
20 refer to the 51 conjugated compounds that comprise <sup>193</sup>mPt  
conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through  
SEQ ID NO:54, respectively.

Compounds 2-197Hg, 3-197Hg and 5-197Hg to 54-197Hg refer to the 51 conjugated compounds that comprise <sup>197</sup>Hg conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-43K, 3-43K and 5-43K to 54-43K refer to the 51 conjugated compounds that comprise <sup>43</sup>K conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-<sup>52</sup>Fe, 3-<sup>52</sup>Fe and 5-<sup>52</sup>Fe to 54-<sup>52</sup>Fe refer to the 51 conjugated compounds that comprise <sup>52</sup>Fe conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

35           Compounds 2-<sup>57</sup>Co, 3-<sup>57</sup>Co and 5-<sup>57</sup>Co to 54-<sup>57</sup>Co refer  
to the 51 conjugated compounds that comprise <sup>57</sup>Co conjugated to

SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-67Ga, 3-67Ga and 5-67Ga to 54-67Ga refer to the 51 conjugated compounds that comprise <sup>67</sup>Ga conjugated to  
5 SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-68Ga, 3-68Ga and 5-68Ga to 54-68Ga refer to the 51 conjugated compounds that comprise <sup>68</sup>Ga conjugated to  
10 SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-77Br, 3-77Br and 5-77Br to 54-77Br refer to the 51 conjugated compounds that comprise <sup>77</sup>Br conjugated to  
SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-81Rb, 3-81Rb and 5-81Rb to 54-81Rb refer to the 51 conjugated compounds that comprise <sup>81</sup>Rb conjugated to  
15 SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-81mKr, 3-81mKr and 5-81mKr to 54-81mKr  
20 refer to the 51 conjugated compounds that comprise <sup>81m</sup>Kr conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-87mSr, 3-87mSr and 5-87mSr to 54-87mSr refer to the 51 conjugated compounds that comprise <sup>87m</sup>Sr  
25 conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-99mTc, 3-99mTc and 5-99mTc to 54-99mTc refer to the 51 conjugated compounds that comprise <sup>99m</sup>Tc  
30 conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-111In, 3-111In and 5-111In to 54-111In refer to the 51 conjugated compounds that comprise <sup>111</sup>In  
conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

Compounds 2-113mIn, 3-113mIn and 5-113mIn to 54-113mIn refer to the 51 conjugated compounds that comprise <sup>113m</sup>In  
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conjugated to SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:5 through SEQ ID NO:54, respectively.

The compounds described in this example are combined with a pharmaceutically acceptable carrier or diluent to produce pharmaceutical compositions according to the present invention. Radiostable compounds described herein are useful in pharmaceutical compositions as therapeutics in the treatment of individuals suspected of suffering from metastasized colorectal cancer including treatment of individuals diagnosed with localized colorectal cancer as a prophylactic/therapeutic before metastasis can be readily detected. When present in therapeutically effective amounts, radioactive compounds described herein are useful in pharmaceutical compositions as therapeutic agents in the treatment of individuals suspected of suffering from metastasized colorectal cancer including treatment of individuals diagnosed with localized colorectal cancer as a prophylactic/therapeutic before metastasis can be readily detected. When present in diagnostically effective amounts, radioactive compounds described herein are useful in pharmaceutical compositions as imaging agents in the diagnosis and identification of metastasized colorectal cancer in individuals.

#### Example 2

One procedure for crosslinking ST receptor ligands which have a free amino group such as ST receptor binding peptides, as for example SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NOS:5-54 to active agents which have a free amino group such as methotrexate, doxorubicin, daunorubicin, cytosinarabioside, cis-platin, vindesine, mitomycin and bleomycin, or alkaline phosphatase, or protein- or peptide-based toxin employs homobifunctional succinimidyl esters, preferably with chain carbon spacers such as disuccinimidyl suberate (Pierce Co, Rockford, IL). This approach of amino group derivatization has been employed successfully to crosslink native ST to biotin and, ultimately, to large agarose beads of micron-scale size, preserving the function of native ST (Hughes, M., et al. (1991)

Biochem. 30:10738; Hakki, S., et al. (1993) *Int. J. Biochem.* 25:557; Almenoff, J.S., et al. (1992) *Mol. Micro.* 8:865; each of which is incorporated herein by reference).

An ST binding ligand with the free amino group such as an ST receptor binding peptide is incubated in the presence of the chemical crosslinking agent and an active agent which have a free amino group in equimolar quantities at room temperature for 15-30 min. Incubation is terminated by separating the reactants by gel permeation chromatography by HPLC. This technique separates the conjugated compounds from free active agents and free ST binding ligands, active agent-active agent conjugates and ST binding ligand-ST binding ligand conjugates. Homogeneous preparations of conjugated through their free amino groups and with a preferred molar ratio of 1:1 are obtained. As indicated above, complexing the free amino group of an ST peptide preserves receptor binding function.

### Example 3

In the event that a cleavable conjugated compound is required, the same protocol as described above may be employed utilizing 3,3'- dithiobis (sulfosuccinimidylpropionate (SPDP); Pierce, IL.). SPDP forms a sulfhydryl group from a free amino group which may be used to conjugate a compound to another free amino group. For example, ST peptides such as SEQ ID NO:2, SEQ ID NO:3, SEQ ID NOS:5-54 are derivatized using established procedures employing N-succinimidyl-3 (2-pyridylthio)-propionate (SPDP, Pharmacia-LKB, NJ). The ST peptide is incubated with a 5-fold molar excess of SPDP for 30 minutes at room temperature. The ST-pyridylthiopropionate conjugate is separated from unreacted reagents by gel permeation chromatography by HPLC. An active agent with a free amino group, such as a protein-based toxin, is prepared for conjugation by reduction with dithiothreitol for 4 hours at room temperature. Reduced active agent is incubated with a 2-fold molar excess of ST receptor ligand-PDP conjugate at pH 8.0 for 36 hours at 4°C. Conjugate compound is purified from unreacted agents by gel permeation chromatography by HPLC.

This protocol for conjugation is particularly useful to conjugate ST peptides to diphtheria toxin A chains and *Pseudomonas* exotoxin as well as ricin toxin A chains (Magerstadt, M. *Antibody Conjugates and Malignant Disease*. (1991) CRC Press, Boca Raton, USA, pp. 110-152; Cawley, D.B. et al. (1980) *Cell* **22**:563; Cumber, A.J., et al. (1985) *Meth. Enz.* **112**:207; Gros, O. (1985) *J. Immunol. Meth.* **81**:283; Worrell, N.R., et al. (1986) *Anti-Cancer Drug Design* **1**:179; Thorpe, P.E. et al. (1987) *Cancer Res.* **47**:5924, each of which is incorporated herein by reference).

#### Example 4

Active agents with a free amino group may be derivatized with SPDP as described above and conjugated with an ST ligand that has a free amino group and that has been modified with the succinimidyl ester of iodoacetic acid (Pierce Co., Rockford, IL) (Magerstadt, M. (1991) *Antibody Conjugates And Malignant Disease*, CRC Press Boca Raton; Cumber, A.J. et al. (1985) *Meth. Enz.* **112**:20, which are incorporated herein by reference). Conjugation relies on the selective reaction of iodoacetyl groups introduced into the amino terminal of the ST ligand with the thiol groups introduced into the active agent. As with the above protocol, this procedure avoids homopolymer formation. However, the product is conjugated through a central thioether linkage which cannot be reduced.

#### Example 5

An ST receptor ligand with a free amino group and active agents with free amino groups may be conjugated through a disulfide bond using iminothiolane (Pierce, Rockford, IL) (Fitzgerald, D.J.P. et al. (1983) *Cell* **32**:607; Magerstadt, M. (1991) *Antibody Conjugates And Malignant Disease*, CRC Press, Boca Raton; Bjorn, M.J., et al. (1985) *Cancer Res.* **45**:1214; Bjorn, M.J., et al. (1986) *Cancer Res.* **46**:3262, which are incorporated herein by reference). The ST receptor ligand with a free amino group is derivatized at the amino terminal with iminothiolane and the active agent is derivatized with SPDP as



described above. Reacting iminothiolane-derivatized ST  
receptor ligand with SPDP-derivatized active agent results in  
conjugation by a reducible disulfide bond. In addition,  
iminothiolane provides the versatility to conjugate these  
5 proteins through bonds other than disulfides. Thus,  
derivatization of active agents with the heterobifunctional  
agent sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane  
(Pierce, Rockford, IL) and reaction with iminothiolane-  
derivatized ST receptor ligand will conjugate these peptides  
10 without formation of disulfides.

#### Example 6

Conjugated compounds according to the invention which  
comprise an active moiety that is a therapeutic agent  
specifically inhibit T84 cells *in vitro*. The following  
15 protocols may be used to demonstrate that the conjugated  
compounds according to the invention which comprise an active  
moiety that is chemotherapeutic or toxin specifically inhibit  
T84 cells *in vitro*. Inhibition of T84 cells is assessed by  
determining the effects of conjugated compounds on the ability  
20 of T84 cells to incorporate <sup>35</sup>S-leucine into protein, <sup>3</sup>H-  
thymidine into DNA, and to form colonies. The assessment of  
protein and DNA synthesis are classical techniques to determine  
the cytotoxicity of conjugated compounds *in vitro*. Inhibition  
of protein synthesis is measured because the toxins used as  
25 active moieties are specific inhibitors of this process.  
Therefore, these assays are the most sensitive measure of  
whether conjugated compounds are binding to and internalized  
into T84 cells. Inhibition of DNA synthesis is measured  
because some chemotherapeutics inhibit DNA synthesis and  
30 further, it is a cytotoxicity assay which correlates closely  
with the reproductive survivability of cells in culture.  
Cytotoxicity, or the disruption of normal cellular metabolic  
processes, may not always directly correlate with cell  
survivability. Therefore, assessment of colony formation will  
35 directly measure the ability of the experimental agents to  
decrease the survivability of tumor cells, which closely  
correlates with the impact of therapeutic agents on tumor

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viability *in vivo*. Controls include performing the same assay using the unconjugated form of the active agent and the unconjugated form of the ST receptor ligand of which the conjugated compound is comprised in place of the conjugated  
5 compound. The results obtained in the test assays and control assays are compared.

Conjugated compounds are assessed for their ability to inhibit protein and DNA synthesis *in vitro* and to inhibit survival and proliferation by measuring colony formation in  
10 monolayer culture by established protocols (Wilson, A.P. (1987) "Cytotoxicity and viability assays", Animal Cell Culture: A Practical Approach. Freshney, R.I., ed. pp. 183-216, IRL Press, Oxford. which is incorporated herein by reference).

To assess the ability of a conjugated compound to  
15 inhibit protein synthesis *in vitro*, cells are plated in 200  $\mu$ l of medium at a sub-confluent density of  $1-2 \times 10^5$  and allowed to attach to form a dividing cell monolayer over 12 hours at 37°C. Subsequently, the media is replaced with 200  $\mu$ l of fresh media containing the appropriate concentration of conjugated  
20 compounds and cells incubated at 37°C for various amounts of time. At the end of the indicated incubation period, cells is washed twice with medium and incubated at 37°C in 0.5 ml of methionine-free medium supplemented with 0.5  $\mu$ Ci of L<sup>35</sup>S-methionine (800 Ci/mmol). After incubation for another 2 hours  
25 at 37°C, the medium is aspirated, cells washed twice with medium containing 1 mg/ml of methionine, and then precipitated in 12% ice-cold TCA. Radioactivity recovered in TCA precipitates by centrifugation is quantified by liquid scintillation spectroscopy. In these studies, cells are  
30 maintained in log growth and assays are performed using triplicate wells. Data is expressed as a percentage of protein synthesis observed in the presence of experimental agents compared to untreated cells.

To assess the ability of a conjugated compound to  
35 inhibit DNA synthesis *in vitro* cells are plated as a subconfluent monolayer and incubated with experimental agents as described above. At the end of the incubation period, cells

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are washed twice and incubated at 37°C in medium containing 2.5  $\mu$ Ci of  $^3\text{H}$ -thymidine (5 Ci/mmol). After incubation for another hour, cells are processed with TCA, precipitates recovered, and radioactivity quantified as described above. As above, cells  
5 are maintained in log growth and assays is performed in triplicate. Data is expressed as a percentage of DNA synthesis observed in the presence of experimental agents compared to untreated cells.

To assess the ability of a conjugated compound to  
10 inhibit survival and proliferation by measuring colony formation in monolayer culture, cells are plated as a sub-confluent monolayer on 25 cm<sup>2</sup> flasks and allowed to attach as described above. The medium is replaced with that containing various concentrations of experimental agents and incubated  
15 with cells for various amounts of time. At the end of the incubation, cells are recovered as a single cell suspension by trypsinization and replated to a density which will yield 100-200 colonies per 6 cm plate. Cells are permitted to grow for 7 days, then fixed in methanol, stained with 1% crystal violet,  
20 and the number of colonies quantified. Assays are performed in duplicate and data is expressed as a percentage of colony formation observed in the presence of experimental agents compared to untreated cells. Results in our laboratory have demonstrated that T84 cells can be placed into single cell  
25 suspensions utilizing trypsin (10  $\mu$ g/ml) with a plating efficiency of 40% and a doubling time of 18 hours.

#### Example 7

Radioactive iodine such as  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$  and  $^{132}\text{I}$ , can be added to an ST receptor binding peptide such as an ST  
30 peptide using a standard protocol well-known to those having ordinary skill in the art (Thompson, M. et al. (1985) *Analytical Biochemistry* 148:26, which is incorporated herein by reference). Radioactive iodine is conjugated directly to an ST peptide such as SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:5 at  
35 tyrosine-5, tyrosine-4 or tyrosine-5, respectively.

Briefly, the ST peptide is produced in bacteria. For example, *E. coli* strain 431 is grown in culture and secretes ST

into this culture. The culture media is then purified using routine techniques. ST can also be made by solid-phase synthesis as has been done previously, using standard techniques. (Dreyfus, L., et al. (1983) *Infect. Immun.* 42:539, 5 which is incorporated herein by reference.

Ten micrograms of ST peptide are reacted with 2 milliCuries of radioactive INa (Amersham Corporation, Massachusetts) in the presence of Iodobeads (Bio Rad Laboratories, CA) and beta-D-glucose. These are reacted for 30 min after which the products are subjected to chromatography on a Sepak reversed-phase cartridge (Millipore Corp., MA) followed by separation on a C<sub>18</sub> reversed-phase column by HPLC using a 20-25% acetonitrile gradient. Conjugated compositions which comprise SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:5 with the 15 radioiodine attached to tyrosine-4 elutes at 45 min. These molecules retain full biochemical and pharmacological activity.

#### Example 8

<sup>125</sup>I is conjugated directly to an ST peptide such as SEQ ID NO:13 at tyrosine-4.

20 SEQ ID NO:13 is produced by solid-phase synthesis as described above. Ten micrograms of SEQ ID NO:13 are reacted with 2 milliCuries of <sup>125</sup>INa (Amersham Corporation, Massachusetts) in the presence of Iodobeads (Bio Rad Laboratories, CA) and beta-D-glucose. These are reacted for 30 min after which the products are subjected to chromatography on a Sepak reversed-phase cartridge (Millipore Corp., MA) followed by separation on a C<sub>18</sub> reversed-phase column by HPLC using a 20-25% acetonitrile gradient. <sup>125</sup>I-SEQ ID NO:13 conjugate with the radioiodine attached to tyrosine-4 elutes at 45 min. This 25 molecule retains full biochemical and pharmacological activity.

Dosing of radioiodine for diagnostic imaging typically requires about 4 milliCuries/patient (Steinstraber, A., et al. (1988) *J. Nucl. Med.* 29:875; Wessels, B.W. and Rogus, R.D. (1984) *Med. Phys.* 11:638; Kwok, C.S., et al. (1985) *Med. Phys.* 35 12:405). For proteins labeled with a specific activity of 2,000 Curies/mmol, such as ST peptide, this would require about

10 micrograms of labeled peptide injected intravenously per patient for diagnostic imaging.

**Example 9**

<sup>131</sup>I is conjugated directly to an ST peptide such as  
5 SEQ ID NO:13 at tyrosine-4.

SEQ ID NO:13 is produced by solid-phase synthesis as described above. Ten micrograms of SEQ ID NO:13 are reacted with 10 milliCuries of <sup>131</sup>INa (Amersham Corporation, Massachusetts) in the presence of Iodobeads (Bio Rad  
10 Laboratories, CA) and beta-D-glucose. These are reacted for 30 min after which the products are subjected to chromatography on a Sepak reversed-phase cartridge (Millipore Corp., MA) followed by separation on a C<sub>18</sub> reversed-phase column by HPLC using a 20-25% acetonitrile gradient. <sup>131</sup>I-SEQ ID NO:13 conjugate with the  
15 radioiodine attached to tyrosine-4 elutes at 45 min. This molecule retains full biochemical and pharmacological activity.

Typically, for radioiodinated antibodies (MW=160,000 Da), about 150 nanomoles of protein (24 milligrams) labeled with a specific activity of 10,000 Curies/mmol are required per  
20 gram of tumor per patient (Humm, J.L. (1986) *J. Nucl. Med.* 27:1490). Thus, for proteins labeled with a specific activity of 2,000 Curies/mmol, with a molecular weight of 2,000 Da, such as ST peptide, about 3 milligrams would be required per gram of tumor per patient for intravenous infusion.

**25 Example 10**

In some embodiments, coupling of ST receptor ligands which have a free amino group, particularly ST receptor binding peptides such as ST peptides, and active agents with a free amino group such as protein-based toxins is performed by  
30 introducing a disulfide bridge between the 2 molecules. This strategy is particularly useful to conjugate ST peptides since the free amino terminal has been shown to be useful as a point of conjugation without affecting ST binding activity. This strategy is particularly useful to conjugate protein-based  
35 toxins since the free amino terminal is available on such molecules and for some conjugated compounds, most notably RTA conjugates, a disulfide bridge which can be reduced to yield

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Disulfide coupling is achieved using established procedures employing the heterobifunctional agent N-succinimidyl-3 (2-pyridyldithio)-proportionate (SPDP, Pharmacia-LKB, Piscataway, NJ) (Magerstadt, M. (1991) *Antibody Conjugates And Malignant Disease*, CRC Press, Boca Raton; Cawley, D.B. et al. (1980) *Cell* **22**:563; Cumber, A.J., et al. (1985) *Meth. Enz.* **112**:20; Gros, O., et al. (1985) *J. Immunol. Meth.* **81**:283; Worrell, N.R., (1986) *Anti-Cancer Drug Design* **1**:19; Thorpe, P.E., et al. (1987) *Cancer Res.* **4**:5924, which are incorporated herein by reference).

In some embodiments, toxins including the A chains of deglycosylated ricin toxin (RTA; Sigma Chemical Co., St. Louis, MO), diphtheria toxin A (DTA; Calbiochem, La Jolla, CA) and *Pseudomonas* exotoxin (PEA) are conjugated to ST peptides to produce conjugated compositions according to the present invention using this procedure. Deglycosylated RTA is employed since the glycosylated form of this toxin exhibits non-specific binding to liver cells. DTA is prepared from diphtheria toxin by an established procedure (Michel, A. and Drykx, J. (1975) *Biochem. Biophys. Acta* 365:15; Cumber, A.J., et al. (1985) *Meth. Enz.* 112:207, both of which are incorporated herein by reference). PEA

35 In some embodiments, ST peptides are conjugated to toxins by this procedure. For example, the ST peptide SEQ ID NO:3 which is produced as described above (see Dreyfus, L., et

al. (1983) *Infec. Immun.* 42:539, which is incorporated herein by reference).

Toxins are prepared for coupling by reduction with 0.1 M dithiothreitol (DTT) for 4 hours at room temperature in 0.4 M Tris-HCl, pH 8.0 and 1mM EDTA. Reduced toxins are desalted on a Sephadex G-25 column equilibrated in TES buffer and mixed with a 2-fold molar excess of ST-PDP. Reactions are adjusted to pH 8.0 with TES and incubated at 4°C for 36 hours. ST peptide-toxin conjugates are purified from unreacted products and homopolymers of ST peptides and toxins by gel filtration on Sephadex G-75 in 20 mM TES, pH 8.0 containing 0.1 M NaCl. Chromatographic fractions are monitored by SDS-PAGE on 10% polyacrylamide gels under non-reducing conditions for the presence of 1:1 conjugates of ST peptides and toxins. Also, these conjugates are analyzed by 10% SDS-PAGE under reducing conditions, to insure that ST and cytotoxins are coupled by a reducible disulfide bond. Molar concentrations of the conjugate are calculated by quantifying radioactivity in these samples.

ST trace labeled with  $^{125}\text{I}$  on tyrosine 4 (10 Ci/mmol) is used in order to follow the conjugate through various separation and chromatographic steps and to enable us to calculate the molar ratio of ST to cytotoxin in the final purified conjugate. ST trace labeled with  $^{125}\text{I}$  is derivatized by incubating 1 mg/ml with a 5-fold molar excess of SPDP for 30 min at room temperature in Na phosphate buffer, pH 7.4. The ST-pyridylthiopropionate (ST-PDP) conjugate is purified from unreacted crosslinking agent by chromatography on Sephadex G-25 equilibrated with 20 mM N-Tris (hydroxymethyl)-methyl-2-aminoethane sulfonic acid (TES) buffer, pH 7.4. Preservation of receptor binding of conjugated ST peptides in human intestinal membranes is determined in competition assays of increasing concentrations of ST-PDP and  $^{125}\text{I}$ -ST ( $5 \times 10^{10}\text{M}$ ), to insure that this process does not destroy the function of the ST receptor ligand.

The above coupling protocol has several advantages for conjugating the various toxins. First, it introduces a

reducible disulfide bridge into the conjugated composition, important for RTA cytotoxicity. Also, this technique avoids the exposure of ST peptide to quantitative reduction with DTT which could interrupt its 3 intrachain disulfide bonds  
5 important for receptor binding activity. In addition, there is a single group available at the amino terminal of ST peptide for derivatization with SPDP and previous experiments have demonstrated that derivatization of that group preserves the binding properties of the ligand. Therefore, other  
10 configurations for conjugation which could result in inactivation of ST are not possible. Furthermore, PEA requires preactivation with DTT to achieve optimum cytotoxicity which will be accomplished utilizing the above protocol.

To produce a functional conjugated compound that comprises a toxin, it is essential that the receptor binding and enzyme activities of the moieties are preserved throughout the process of conjugation. Therefore, once such conjugate compounds are obtained, they are tested for the preservation of those functions. ST receptor binding activity of conjugated compounds is examined in competitive binding assays, as described above. In these studies, increasing concentrations of the conjugated compounds are incubated with a constant concentration ( $5 \times 10^{10}$ M) of  $^{125}$ I-ST and intestinal membranes (50-100  $\mu$ g of protein) to achieve equilibrium. Parallel incubations contain excess ( $5 \times 10^7$ M) unlabeled ST to assess non-specific binding. The concentration-dependent competitive displacement of radiolabeled ST by conjugated compounds is compared to the competitive displacement achieved by native ST. Displacement curves are employed to estimate the affinity of each conjugated compound ( $K_D$ ) and compare that to the affinity of native ST measured by this technique. Control studies include evaluating the ability of unconjugated toxins to compete with native ST for receptor binding. These studies establish that the binding function of ST in the conjugated construct is preserved.

Preservation of toxin activity in conjugated compounds is also assessed. PEA and DTA induce toxicity by catalyzing



the NAD-dependent ADP-ribosylation of elongation factor 2 (EF2), inhibiting protein synthesis. ADP-ribosyl transferase activity is assessed using an established assay (Chung, D.W. and Collier, R.J. *Infect. Immun.* 16:832; Fitzgerald, D.J.P. (1987) *Meth. Enz.* 151:139, which are both incorporated herein by reference). Reactions are conducted in 30 mM Tris-HCl, pH 8.2 containing 40 mM DTT, 50 mCi  $^{14}\text{C}$ -NAD, and 20  $\mu\text{l}$  of rabbit reticulocyte lysate containing elongation factor 2 (EF-2; Promega, Madison, WI) in a total volume of 500  $\mu\text{l}$ . Reactions are initiated by the addition of lysate, incubated for 30 minutes at 37°C, and terminated by the addition of ice-cold 12% TCA. Radioactivity in protein precipitates collected by centrifugation is quantified by liquid scintillation spectroscopy. The ability of the conjugated compounds that comprise DTA or PEA to catalyze the transfer of labeled ADP-ribose to EF-2 is compared to that catalyzed by similar quantities of unconjugated toxins. Control experiments include examining the ability of unconjugated toxins or ST to catalyze ADP-ribose transfer and the effects of ST on the enzymatic activity of unconjugated cytotoxins.

RTA inhibits protein synthesis by catalytically inactivating the 60S ribosomal subunit. The catalytic activity of conjugated compounds that comprise RTA is assessed by its ability to inhibit protein synthesis in cell-free assays using established procedures (Leonard, J.E. et al. (1985) *Cancer Res.* 45:5263 which is incorporated herein by reference). Assays contain 35  $\mu\text{l}$  of nuclease-treated rabbit reticulocyte lysates, 1  $\mu\text{l}$  of 1mM mixed amino acids deficient in methionine, 2  $\mu\text{l}$  of Brome mosaic RNA (Promega, Madison, WI) at 0.5  $\mu\text{g}/\mu\text{l}$ , 7  $\mu\text{l}$  of sterile water or conjugate solution, and 5  $\mu\text{Ci}$  of  $^{35}\text{S}$ -methionine in a total volume of 50  $\mu\text{l}$ . Reactions will be initiated by the addition of lysate, incubated at 30°C for 30 minutes, and terminated by the use of addition of 12% TCA. Radioactivity in protein precipitates collected by centrifugation is quantified by liquid scintillation spectroscopy. Control experiments include examining the ability of unconjugated RTA or ST peptide to inhibit cell-free protein synthesis and the effects of ST

peptide on the inhibitory activity of the unconjugated cytotoxin.

**Example 11**

Methotrexate is linked to SEQ ID NO:12 by the  
5 homobifunctional crosslinker succinimidyl esters with long  
chain carbon spacers such as disuccinimidyl suberate (Pierce,  
IL). SEQ ID NO:12 is incubated in the presence of the chemical  
crosslinking agent and methotrexate in equimolar quantities at  
room temperature for 15-30 min. Incubation is terminated by  
10 separating the reactants by gel permeation chromatography by  
HPLC. This technique separates the methotrexate/SEQ ID NO:12  
conjugates from free drug, free ST peptide, drug-drug  
conjugates and ST peptide-ST peptide conjugates. Homogeneous  
preparations of SEQ ID NO:12-methotrexate conjugates coupled  
15 through their free amino groups and with a preferred molar  
ratio of 1:1 are obtained. Complexing the free amino group of  
ST preserves receptor binding function.

**Example 12**

<sup>111</sup>In is coupled to SEQ ID NO:37 with functional amino  
20 groups using a chelator. The ST peptide has a free amino  
function at the amino terminal which may be modified without  
altering the ST receptor binding activity of the ST peptide.  
<sup>111</sup>In is rapidly and potently chelated by either EDTA  
(ethylenediaminetetraacetic acid) or DTPA  
25 (diethylenetriaminepentaacetic acid). DTPA is preferred over  
EDTA because the latter may be more unstable *in vivo*. The  
<sup>111</sup>In-DTPA is converted to a mixed N-hydroxysuccinimide ester  
which is reactive with free amino groups, mixed with ST, and  
the reaction products, including <sup>111</sup>In-SEQ ID NO:37 separated by  
30 HPLC (Bremer, K.H. and Schwarz, A. (1987) in *Safety And  
Efficacy Of Radiopharmaceuticals*. Kristensen, K. and  
Norbygaard, E., Eds. Martinus Nijhoff, Dordrecht, The  
Netherlands, P. 43; Krejcarek, G.E., and Tucker, K.L. (1977)  
*Biochem. Biophys. Res. Commun.* **77**:581; Paxton, R.J., et al.  
35 (1985) *Cancer Res.* **45**:5694; Richardson, A.P., et al. (1986)  
*Nucl. Med. Biol.* **14**:569, which are each incorporated herein by  
reference).

**Example 13**

<sup>99m</sup>Tc can be conjugated to SEQ ID NO:46 using an approach which is similar to that for indium. Thus, technetium can be chelated by DTPA which is converted to an anhydride, such as N-hydroxysuccinimide anhydride, and reacted with SEQ ID NO:46. The ST-technetium conjugate can then be separated using HPLC (Magerstadt, M. (1991) *Antibody Conjugates And Malignant Disease* CRC Press, Boca Raton; Eckelman, W.C. and Paik, C.H. (1986) *Nucl. Med. Biol.* **14**:569)

**Example 14**

Diphtheria toxin A chain (DTA) is prepared from native diphtheria toxin by standard techniques. SEQ ID NO:22 is coupled to N-succinimidyl-3(2-pyridyldithio)-propionate (SPDP, Pharmacia-LKB, Piscataway, NJ) and the SEQ ID NO:22-PDP conjugate is purified by HPLC by established procedures. DTA is reduced with dithiothreitol and incubated with SEQ ID NO:22-PDP. DTA-SEQ ID NO:22 is purified after conjugation using HPLC.

**Example 15**

*Pseudomonas* Exotoxin is prepared from native sources by standard techniques. SEQ ID NO:54 is coupled to N-succinimidyl-3(2-pyridyldithio)-propionate (SPDP, Pharmacia-LKB, Piscataway, NJ) and the SEQ ID NO:54-PDP conjugate is purified by HPLC by established procedures. *Pseudomonas* Exotoxin is reduced with dithiothreitol and incubated with SEQ ID NO:54-PDP. *Pseudomonas* Exotoxin-SEQ ID NO:54 is purified after conjugation using HPLC.

**Example 16**

Doxorubicin is linked to SEQ ID NO:54 by the homobifunctional crosslinker succinimidyl esters with long chain carbon spacers such as disuccinimidyl suberate (Pierce, IL). SEQ ID NO:54 is incubated in the presence of the chemical crosslinking agent and doxorubicin in equimolar quantities at room temperature for 15-30 min. Incubation is terminated by separating the reactants by gel permeation chromatography by HPLC. This technique separates the doxorubicin/SEQ ID NO:54 conjugates from free doxorubicin, free ST peptide, drug-drug



## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Waldman, Scott A.
- (ii) TITLE OF INVENTION: ST Receptor Binding Compounds and Methods of Using the Same
- (iii) NUMBER OF SEQUENCES: 54
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Woodcock Washburn Kurtz Mackiewicz & Norris
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  - (C) CITY: Philadelphia
  - (D) STATE: Pennsylvania
  - (E) COUNTRY: USA
  - (F) ZIP: 19103
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: DeLuca, Mark
  - (B) REGISTRATION NUMBER: 33,229
  - (C) REFERENCE/DOCKET NUMBER: TJU-903
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 215-568-3100
  - (B) TELEFAX: 215-568-3439

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 57 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: both
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 1..57
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

AAC AAC ACA TTT TAC TGC TGT GAA CTT TGT TGT AAT CCT GCC TGT GCT      48
Asn Asn Thr Phe Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Ala
  1               5               10               15

GGA TGT TAT
Gly Cys Tyr

```

57

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Asn Asn Thr Phe Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Ala  
 1                      5                      10                      15  
 Gly Cys Tyr

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Asn Thr Phe Tyr Cys Cys Glu Leu Cys Cys Tyr Pro Ala Cys Ala Gly  
 1                      5                      10                      15  
 Cys Asn

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: both

## (ii) MOLECULE TYPE: cDNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..57

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AAT AGT AGC AAT TAC TGC TGT GAA TTG TGT TGT AAT CCT GCT TGT AAC  
 Asn Ser Ser Asn Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Asn  
 1                      5                      10                      15  
 GGG TGC TAT  
 Gly Cys Tyr

48

57

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Asn Ser Ser Asn Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Asn  
1 5 10 15  
Gly Cys Tyr

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 15 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Pro Asn Thr Cys Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys  
1 5 10 15

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Asn Asn Thr Phe Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Ala  
1 5 10 15  
Gly Cys

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Asn Thr Phe Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Ala Gly  
1 5 10 15  
Cys

## (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 16 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Thr Phe Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Ala Gly Cys  
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Phe Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Ala Gly Cys  
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Ala Gly Cys  
1 5 10

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Ala Gly Cys  
1 5 10

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:



Asn Thr Phe Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Ala Gly  
1 5 10 15

Cys Tyr

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Thr Phe Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Ala Gly Cys  
1 5 10 15

Tyr

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 16 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Phe Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Ala Gly Cys Tyr  
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 15 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Ala Gly Cys Tyr  
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 14 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

Figure 1 consists of 11 bar charts, labeled (a) through (k), each representing a different variable. The x-axis for all charts is divided into two main sections: '2008' and '2012'. Within each year section, there are sub-categories for 'Male', 'Female', and 'Total'. The y-axis represents the percentage of respondents, ranging from 0% to 100%.

- (a) Age:** Shows percentages for 18-24, 25-34, 35-44, 45-54, 55-64, 65-74, and 75+.
- (b) Sex:** Compares Male and Female respondents.
- (c) Education:** Compares High School, College, and Graduate.
- (d) Income:** Compares Less than \$10,000, \$10,000-\$20,000, \$20,000-\$30,000, \$30,000-\$40,000, \$40,000-\$50,000, \$50,000-\$60,000, \$60,000-\$70,000, \$70,000-\$80,000, \$80,000-\$90,000, \$90,000-\$100,000, and More than \$100,000.
- (e) Employment:** Compares Full-time, Part-time, and Unemployed.
- (f) Religion:** Compares Catholic, Protestant, Jewish, Muslim, and Other.
- (g) Political Party:** Compares Democrat, Republican, and Independent.
- (h) Marital Status:** Compares Married, Divorced, Widowed, and Single.
- (i) Number of Children:** Compares 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100.
- (j) Number of Siblings:** Compares 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100.
- (k) Attitudes towards gay, lesbian, and transgender people:** Compares Strongly Oppose, Oppose, Neutral, Support, and Strongly Support.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Ala Gly Cys Tyr  
1 5 10

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Asn Thr Phe Tyr Cys Cys Glu Leu Cys Cys Tyr Pro Ala Cys Ala Gly  
1 5 10 15

Cys

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 16 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Thr Phe Tyr Cys Cys Glu Leu Cys Cys Tyr Pro Ala Cys Ala Gly Cys  
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 15 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Phe Tyr Cys Cys Glu Leu Cys Cys Tyr Pro Ala Cys Ala Gly Cys  
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 14 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide



(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Tyr Cys Cys Glu Leu Cys Cys Tyr Pro Ala Cys Ala Gly Cys Asn  
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 14 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Cys Cys Glu Leu Cys Cys Tyr Pro Ala Cys Ala Gly Cys Asn  
1 5 10

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Asn Ser Ser Asn Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr  
1 5 10 15  
Gly Cys

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ser Ser Asn Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly  
1 5 10 15  
Cys

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 16 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Ser Asn Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys  
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 15 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Asn Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys  
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 14 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys  
1 5 10

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 13 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys  
1 5 10

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Ser Ser Asn Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly

003493 4360

1

5

10

15

Cys Tyr

## (2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Ser Asn Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys  
1 5 10 15

Tyr

## (2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 16 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Asn Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr  
1 5 10 15

## (2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 15 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr  
1 5 10 15

## (2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 14 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr  
1 5 10

(2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Asn Thr Phe Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Ala Gly  
1 5 10 15

Cys Tyr

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Asn Thr Phe Tyr Cys Cys Glu Leu Cys Cys Ala Pro Ala Cys Ala Gly  
1 5 10 15

Cys Tyr

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Asn Thr Phe Tyr Cys Cys Glu Leu Cys Cys Asn Ala Ala Cys Ala Gly  
1 5 10 15

Cys Tyr

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

003447 "E2642/60

Cys

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:



Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Ala Gly Cys  
1 5 10

(2) INFORMATION FOR SEQ ID NO:46:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 25 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Gln Ala Cys Asp Pro Pro Ser Pro Pro Ala Glu Val Cys Cys Asp Val  
1 5 10 15

Cys Cys Asn Pro Ala Cys Ala Gly Cys  
20 25

(2) INFORMATION FOR SEQ ID NO:47:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 16 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Ile Asp Cys Cys Ile Cys Cys Asn Pro Ala Cys Phe Gly Cys Leu Asn  
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:48:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Ser Ser Asp Trp Asp Cys Cys Asp Val Cys Cys Asn Pro Ala Cys Ala  
1 5 10 15

Gly Cys

(2) INFORMATION FOR SEQ ID NO:49:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

[illegible]

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Asn Ser Ser Asn Tyr Cys Cys Glu Leu Cys Cys Tyr Pro Ala Cys Thr  
1 5 10 15

Gly Cys Tyr

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 13 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Cys Cys Asp Val Cys Cys Asn Pro Ala Cys Thr Gly Cys  
1 5 10

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 14 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Cys Cys Asp Val Cys Cys Tyr Pro Ala Cys Thr Gly Cys Tyr  
1 5 10

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 14 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Cys Cys Asp Leu Cys Cys Asn Pro Ala Cys Ala Gly Cys Tyr  
1 5 10

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 14 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Cys Cys Gln Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr  
1 5 10

(2) INFORMATION FOR SEQ ID NO:54:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 15 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Cys Cys Gln Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr  
1 5 10 15

Pro Gly Thr Cys Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys

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## CLAIMS

1. A conjugated compound comprising:  
a) a ST receptor binding moiety; and,  
b) an active moiety;
- 5 wherein said active moiety is a radiostable active agent.
2. The compound of claim 1 wherein said ST receptor binding moiety is a peptide.
3. The compound of claim 1 wherein said ST receptor binding moiety is selected from the group consisting of: SEQ ID  
10 NO:2, SEQ ID NO:3, SEQ ID NOS:5-54 and fragments and derivatives thereof.
4. The compound of claim 1 wherein said ST receptor binding moiety is selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:54.
- 15 5. The compound of claim 1 wherein said an active moiety is a therapeutic agent.
6. The compound of claim 1 wherein said an active moiety is selected from the group consisting of: methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4  
20 fluorouracil, melphalan, chlorambucil, *cis*-platinum, vindesine, mitomycin, bleomycin, purothionin, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, *Pseudomonas* exotoxin, diphtheria toxin, *Clostridium perfringens* phospholipase C, bovine pancreatic ribonuclease, pokeweed  
25 antiviral protein, abrin, abrin A chain, cobra venom factor, gelonin, saporin, modeccin, viscumin, volkensin, alkaline phosphatase, nitroimidazole, metronidazole and misonidazole.
7. The compound of claim 1 wherein:

a) said ST receptor binding moiety is selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NOS:5-54 and fragments and derivatives thereof;

b) said an active moiety is selected from the group consisting of: methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, *cis*-platinum, vindesine, mitomycin, bleomycin, purothionin, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, *Pseudomonas* exotoxin, diphtheria toxin, *Clostridium perfringens* phospholipase C, bovine pancreatic ribonuclease, pokeweed antiviral protein, abrin, abrin A chain, cobra venom factor, gelonin, saporin, modeccin, viscumin, volkensin, alkaline phosphatase, nitroimidazole, metronidazole and misonidazole.

8. The compound of claim 1 wherein said an active moiety is selected from the group consisting of: methotrexate, doxorubicin, daunorubicin, cytosinarabioside, *cis*-platin, vindesine, mitomycin and bleomycin, alkaline phosphatase, ricin A chain, *Pseudomonas* exotoxin and diphtheria toxin.

9. The compound of claim 1 wherein:

a) said ST receptor binding moiety is selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:54; and

b) said an active moiety is selected from the group consisting of: methotrexate, doxorubicin, daunorubicin, cytosinarabioside, *cis*-platin, vindesine, mitomycin and bleomycin, alkaline phosphatase, ricin A chain, *Pseudomonas* exotoxin and diphtheria toxin.

10. A pharmaceutical composition comprising:

a) a pharmaceutically acceptable carrier or diluent, and,

b) a conjugated compound according to claim 1.

11. A method of treating an individual suspected of suffering from metastasized colorectal cancer comprising the steps of administering to said individual a pharmaceutical composition according to claim 10.
- 5 12. A pharmaceutical composition comprising:  
a) a pharmaceutically acceptable carrier or diluent, and,  
b) conjugated compound comprising:  
i) a ST receptor binding moiety; and,  
10 ii) an active moiety;  
wherein said active moiety is a radioactive agent and said conjugated compound is present in an amount effective for therapeutic or diagnostic use in a humans suffering from colorectal cancer.
- 15 13. The pharmaceutical composition of claim 12 wherein said active moiety is selected from the group consisting of:  $^{47}\text{Sc}$ ,  $^{67}\text{Cu}$ ,  $^{90}\text{Y}$ ,  $^{109}\text{Pd}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{199}\text{Au}$ ,  $^{211}\text{At}$ ,  $^{212}\text{Pb}$ ,  $^{212}\text{B}$ ,  $^{32}\text{P}$  and  $^{33}\text{P}$ ,  $^{71}\text{Ge}$ ,  $^{77}\text{As}$ ,  $^{103}\text{Pb}$ ,  $^{105}\text{Rh}$ ,  $^{111}\text{Ag}$ ,  $^{119}\text{Sb}$ ,  $^{121}\text{Sn}$ ,  $^{131}\text{Cs}$ ,  $^{143}\text{Pr}$ ,  $^{161}\text{Tb}$ ,  $^{177}\text{Lu}$ ,  $^{191}\text{Os}$ ,  $^{193}\text{Pt}$  and  $^{197}\text{Hg}$ .
- 20 14. The pharmaceutical composition of claim 12 wherein said active moiety is selected from the group consisting of:  $^{43}\text{K}$ ,  $^{52}\text{Fe}$ ,  $^{57}\text{Co}$ ,  $^{67}\text{Cu}$ ,  $^{67}\text{Ga}$ ,  $^{68}\text{Ga}$ ,  $^{77}\text{Br}$ ,  $^{81}\text{Rb}/^{81\text{M}}\text{Kr}$ ,  $^{87\text{M}}\text{Sr}$ ,  $^{99\text{M}}\text{Tc}$ ,  $^{111}\text{In}$ ,  $^{113\text{M}}\text{In}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{127}\text{Cs}$ ,  $^{129}\text{Cs}$ ,  $^{131}\text{I}$ ,  $^{132}\text{I}$ ,  $^{197}\text{Hg}$ ,  $^{203}\text{Pb}$  and  $^{206}\text{Bi}$ .
15. The pharmaceutical composition of claim 12 wherein  
25 said ST receptor binding moiety is a peptide.
16. The pharmaceutical composition of claim 12 wherein said ST receptor binding moiety is selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NOS:5-54 and fragments and derivatives thereof.
- 30 17. The pharmaceutical composition of claim 12 wherein said ST receptor binding moiety is selected from the group

consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:54.

18. The pharmaceutical composition of claim 12 wherein said ST receptor binding moiety is selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:54. said active moiety is selected from the group consisting of:  $^{47}\text{Sc}$ ,  $^{67}\text{Cu}$ ,  $^{90}\text{Y}$ ,  $^{109}\text{Pd}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{199}\text{Au}$ ,  $^{211}\text{At}$ ,  $^{212}\text{Pb}$ ,  $^{212}\text{B}$ ,  $^{32}\text{P}$  and  $^{33}\text{P}$ ,  $^{71}\text{Ge}$ ,  $^{77}\text{As}$ ,  $^{103}\text{Pb}$ ,  $^{105}\text{Rh}$ ,  $^{111}\text{Ag}$ ,  $^{119}\text{Sb}$ ,  $^{121}\text{Sn}$ ,  $^{131}\text{Cs}$ ,  $^{143}\text{Pr}$ ,  $^{161}\text{Tb}$ ,  $^{177}\text{Lu}$ ,  $^{191}\text{Os}$ ,  $^{193}\text{Pt}$  and  $^{197}\text{Hg}$ .
- 10 19. The pharmaceutical composition of claim 12 wherein said ST receptor binding moiety is selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:54.  $^{43}\text{K}$ ,  $^{52}\text{Fe}$ ,  $^{57}\text{Co}$ ,  $^{67}\text{Cu}$ ,  $^{67}\text{Ga}$ ,  $^{68}\text{Ga}$ ,  $^{77}\text{Br}$ ,  $^{81}\text{Rb}/^{81\text{M}}\text{Kr}$ ,  $^{87\text{M}}\text{Sr}$ ,  $^{99\text{M}}\text{Tc}$ ,  $^{111}\text{In}$ ,  $^{113\text{M}}\text{In}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{127}\text{Cs}$ ,  $^{129}\text{Cs}$ ,  $^{131}\text{I}$ ,  $^{132}\text{I}$ ,  $^{197}\text{Hg}$ ,  $^{203}\text{Pb}$  and  $^{206}\text{Bi}$ .
- 15 20. A method of radioimaging metastasized colorectal cancer cells comprising the steps of administering to an individual a pharmaceutical composition comprising:
- 20 and,
- a) a pharmaceutically acceptable carrier or diluent,
- b) conjugated compound comprising:
- i) a ST receptor binding moiety; and,
- ii) an active moiety;
- wherein said active moiety is a radioactive agent and said
- 25 conjugated compound is present in an amount effective for diagnostic use in a humans suffering from colorectal cancer.
21. A method of treating an individual suspected of suffering from metastasized colorectal cancer comprising the steps of administering to said individual a pharmaceutical
- 30 composition comprising:
- a) a pharmaceutically acceptable carrier or diluent,
- and,
- b) conjugated compound comprising:

- i) a ST receptor binding moiety; and,
- ii) an active moiety;

wherein said active moiety is a radiostable agent or radioactive agent and said conjugated compound is present in an amount effective for therapeutic or diagnostic use in a humans suffering from colorectal cancer.

22. A method of delivery a nucleic acid molecule to intestinal tract cells of an individual comprising the steps of administering to said individual a pharmaceutical composition comprising:

- a) a pharmaceutically acceptable carrier or diluent, and,
- b) a composition comprising:
  - i) a ST receptor ligand; and,
  - ii) a nucleic acid molecule.



## ABSTRACT

Conjugated compounds which comprises an ST receptor binding moiety and a radiostable active moiety are disclosed. Pharmaceutical compositions comprising a pharmaceutically acceptable carrier or diluent, and a conjugated compound which comprises an ST receptor binding moiety and a radiostable active moiety or an ST receptor binding moiety and a radioactive active moiety are disclosed. Methods of treating an individual suspected of suffering from metastasized colorectal cancer comprising the steps of administering to said individual a pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent, and a therapeutically effective amount of a conjugated compound which comprises an ST receptor binding moiety and a radiostable active moiety or an ST receptor binding moiety and a radiostable active moiety are disclosed. Methods of radioimaging metastasized colorectal cancer cells comprising the steps of first administering to an individual suspected of having metastasized colorectal cancer cells, a pharmaceutical composition that comprises a pharmaceutically acceptable carrier or diluent, and conjugated compound that comprises an ST receptor binding moiety and a radioactive active moiety wherein the conjugated compound is present in an amount effective for diagnostic use in humans suffering from colorectal cancer and then detecting the localization and accumulation of radioactivity in the individual's body are disclosed.

## COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and

I verily believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: **ST RECEPTOR BINDING COMPOUNDS AND METHODS OF USING THE SAME** the specification of which:

(xx) is attached hereto.

( ) was filed on \_\_\_\_\_ as Application Serial No. \_\_\_\_\_ and was amended on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to be material to the patentability of this application in accordance with 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of any application on which priority is claimed:

Country	Number	Date Filed	Priority Claimed
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to be material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status (patented, pending)
_____	_____	_____
_____	_____	_____
_____	_____	_____

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: Philip S. Johnson and Mark DeLuca, Registration Nos. 27,200 and 33,229 of the firm of WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS, One Liberty Place - 46th Floor, Philadelphia, Pennsylvania 19103, and

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both; under Section 1001 of Title 18 of the

United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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